

From the review of the original NDA, it is reasonable to conclude that efficacy was not compromised by the time interval between product activation and administration. In preclinical animal studies, DMP-115 remains efficacious in producing echocardiographic demonstration of left ventricles and of myocardial perfusion following activation up to four days post-preparation. The responses produced at later stages following preparation are comparable to the response obtained immediately post preparation despite the fact that the number of particles per ml remaining was less. This suggests that a lower concentration of DMP 115 might be sufficient to obtain clinical efficacy (page 83 of my original review).

Injecting DMP 115 five minutes after product activation led to the injection of DMP 115 containing a high amount of PFP gas with the resultant increase in pulmonary insult. The pulmonary insult is absent when the activated product is allowed to sit for a longer time period.

To this end, I am of the opinion that the time interval between activation and injection is a labeling issue. The agency should encourage the sponsor to stipulate a minimum time interval between product activation and administration.

The results of the study (UOAW-156) conducted to assess the risk of arrhythmias are deemed adequate.

The results of the reproductive toxicology studies are deemed adequate. I recommend approval of DuPont's request for Pregnancy category B language.

Overall, the sponsor's responses have adequately addressed the agency's concern about the risk of arrhythmias, and the fact that the original NDA submission did not use the final to be marketed formulation for the conduct of the reproductive toxicology studies. The studies addressing the risk for the occurrence of micropulmonary emboli in a chronically compromised pulmonary vasculature disease model and for an *in vivo* evaluation of the potential for clumping, aggregation or coalescence in the systemic circulation are deemed inadequate. Therefore, the sponsor should be asked to repeat these studies.

Conclusions: The application is approvable pending successful resolution of issues identified in this review. We believe that the assessment of the risk for the occurrence of micropulmonary emboli in chronically compromised pulmonary vasculature should be addressed prior to the product approval. Evaluation of the potential for clumping, aggregation or coalescence in the systemic circulation can be addressed as a phase 4 commitment. Based on results of studies submitted, the sponsor should be encouraged to stipulate in the product package insert a minimum time interval between product activation and administration.

1 pages redacted from this section of
the approval package consisted of draft labeling

(P.16)

Nursing Mothers

Draft Labeling

Pediatric Use

DRAFT Labeling

RECOMMENDATIONS: From pre-clinical pharmacology and toxicology perspective, the application is approvable pending successful resolution of issues identified in this review. We believe that the assessment of the risk for the occurrence of microplumony emboli in chronically compromised pulmonary vasculature should be addressed prior to the product approval. Evaluation of the potential for clumping, aggregation or coalescence in the systemic circulation can be addressed as a phase 4 commitment. Based on results of studies submitted, the sponsor should be encouraged to stipulate in the product package insert a minimum time interval between product activation and administration.

External comments to sponsor:

1)

Both the dog cardiovascular study and the dose response of DMP 115 and perfluoropropane in conscious rat (DRR 2000-01 and DRR 2000-02) confirmed the importance of PFP gas in the manifestation of the overt signs of toxicity elicited by the animals. However, the studies main conclusion that the adverse pulmonary and cardiovascular events are attributable to the PFP component of the formulation has not answered the primary question posed by the agency concerning the safety of this product in animal models with compromised pulmonary functions. In order to resolve this deficiency, we recommend:

Completion of a study to be conducted in an animal model with compromised pulmonary circulation (pulmonary hypertension) in order to assess possible effects of DMP 115 in target populations.

2)

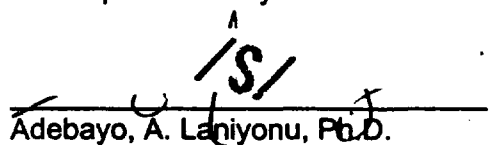
We noted the results of the study assessing the microvascular rheology of Definity microbubbles (DRR 98-08). We agree with the study's conclusion that Definity microbubbles transit through normal capillaries as a single microbubble, and do not cause any capillary or other microvascular obstruction after intravenous administration and pulmonary passage. However, we believe that the study should have been conducted with the injection given intra-arterially. Such an approach would have been helpful for risk identification especially in cases of

patients with right-to-left, bidirectional, or transient right-to left cardiac shunt where phospholipid-encapsulated microbubbles can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation. In order to resolve this deficiency, the following are requested:


An in vivo evaluation of the potential for clumping, aggregation or coalescence in the systemic circulation with Definity administered intra-arterially.

We suggest that you submit the protocols for such studies prior to study initiation.

Reviewer's signature:


Adebayo, A. Lanijonu, Ph.D.
Date 06/06/2000

Team Leader Concurrence:


Nakissa Sadrieh, Ph.D.
Date 6/6/00

Appendix/attachments:

CC:

Original NDA
DIVISION FILES
HFD-160/LANIYONU/SADRIEH
HFD-160/ZOLMAN/JONES
HFD-160/CHO

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Ultrasound contrast agent, Perfluoropropane

Reviewer Name: Adebayo Laniyonu, Ph.D.
Division Name: Medical Imaging and Radiopharmaceutical Drug Products
HFD#: 160
Review Completion Date:

Review number: #1

NDA number: 21-064

Serial number: 000, Pharmacology copies: Volumes 1.1, 1.9 - 1.31,
BP, NC
IND N-070 IT

Date: 09/12/98

Type of submission: N

Information to sponsor: Yes (X) No ()

Sponsor (or agent):

DuPont Pharmaceuticals Company
Medical Imaging Division
331 Treble Cove Road
North Billerica, MA 01862

Manufacturer (drug substance):

Drug:

Code Names: DMP 115, SG897, MRX-115 and Aerosomes
Generic Name: Perfluoropropane
Trade Name: Definity™
Chemical Name: 1,1,1,2,2,3,3,3-Octafluoropropane
CAS Registry Number: 76-19-7
Molecular Weight: 188.02 g/mol

Relevant INDs/NDAs/DMFs:

IND # DMFs #

Drug Class:

Ultrasound Contrast agent

Indication:

Echocardiography

Clinical formulation:

Vial Component	Concentration/mL
Sodium Chloride	6.8 mg
Propylene Glycol	103.5 mg
Glycerin	126.2 mg
Water for Injection, USP	
*Lipid Blend	0.75 mg
Sodium Hydroxide, NF	As needed to adjust pH
Hydrochloric Acid, NF	As needed to adjust pH
Perfluoropropane Gas	% in the Headspace
Injectate Characteristics	
Perfluoropropane Gas	0.15±0.10 mL
Number of Microbubbles (1µm-<10µm) (Clinically useful Range)	~1.2 x 10 ¹⁰

According to the sponsor, DMP 115 is the active ingredient. The drug product contains PFP, a blend of three lipids:

(DPPC), (MPEG5000 DPPE), saline, propylene glycol and glycerin. Following agitation, a milky white microbubble suspension of PFP gas with a mean bubble diameter of 1.5 µm and a count greater than 6 x 10⁷ per mL is formed.

There have been 3 manufacturing routes for lipid blend:

Route1: The three lipids were mixed in , followed by . This procedure was used in the manufacture of drug product Lot No. 744-71-0001 utilized for the preclinical studies and Phase 1 single-dose human trial.

Route 2: The three lipids were mixed in water, followed by . This procedure was used in the manufacture of drug product Lot No. 744-71-002 and 744-71-004 utilized for Phases 2 and 3 clinical trials.

Route 3: The three lipids were dissolved in a mixture of and . After concentration,) is added, and the solids isolated by . followed by . Lots No. 4505Z, PP97A-034, and PP97A-024 produced were used for the core toxicology studies (with the exception of reproductive and developmental toxicity studies) and Phase 3 clinical trials. The DuPont product, DPM-115, which is the "to be marketed" formulation is prepared by route 3 method of blending lipids.

Route of administration: Intravenous

Previous clinical experience: See medical officer review. The recommended dose as described in the label is as a single dose of 10 μ L/kg by slow i.v bolus injection over 30-60 seconds, followed by a 10 mL saline flush. A second, 10 μ L/kg dose may be administered to prolong optimal imaging. The sponsor also suggested that Definity™ be administered via an i.v infusion of 1.3 mL added to 50 mL of preservative-free saline initiated at 4.0 mL/minute and titrated as necessary to achieve optimal image. The highest dose tested during clinical trial with the final formulation was 50 μ L/kg.

Disclaimer use of sponsor's material:

Sponsor submitted texts were utilized in the preparation of this review. They will be identified as quotes

Introduction and drug history:

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed as a contrast-enhancing agent for clinical ultrasound. The sponsor stated that the submitted data support the use of Definity for contrast-enhanced echocardiographic imaging of cardiac structure :

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator. Perfluoropropane is used in the ophthalmic industry to fill the vitreous cavity in the eye following retinal surgery. Changes were made in the clinical manufacturing process as discussed under formulation. The IND studies for this product, IND # were reviewed by Drs. Dundore and Sadrieh. **Those studies that were reviewed by them will not be reviewed again. However, a summary of the studies will be provided and the initial reviewer identified.**

Studies reviewed for the NDA:

RDR 98-26: Correlation of DMP 115 image intensity with bubble concentration. Vol.1.9. pp. 236-250

RDR 98-20: Determination of the optimal dose of DMP 115 for assessment of ventricular opacification and second harmonic imaging in the anesthetized dog. Vol. 1.9 pp. 292-307

RDR98-21: Assessment of DMP 115, 4 days post-preparation on ventricular opacification and second harmonic imaging in the anesthetized dog. Vol.1.10 pp. 2-23

RDR 98-15: Comparison of stability of DMP 115 ultrasound signal in saline or canine whole blood using clinically relevant power levels of ultrasound. Study was conducted by DuPont Pharmaceuticals, Bilerica, MA. Vol.1.9 pp. 214-235

RDR 98-16: An in vitro assessment of DMP 115 fragility under exposure to ultrasound at different power levels. Study was conducted by DuPont Pharmaceuticals, Bilerica, MA. Vol.1.9 pp. 251-273

RDR98-22: Comparison of DMP 115 manufactured via an process for assessment of ventricular opacification and second harmonic imaging in the anesthetized dog. Vol.1.10 pp. 24-46

RDR98-23: Dose optimization and comparison of various modes of administration on ventricular opacification and second harmonic imaging in the anesthetized dog. Vol.1.10 pp.47-66

RDR-98-19: Determination of the ability of DMP 115 to detect myocardial perfusion defects in a canine model of occlusion/reperfusion using gated second harmonic imaging. Vol.1.10 pp. 84-96.

RDR 98-27: Assessment of DMP 115 responses to pressure and ultrasound in normal saline at 37°C. A comparison of six qualification lots manufactured at two sites. Vol.1.9 pp. 170-179

RDR98-24: Determination of effect of DMP 115 bubble size on ventricular opacification and second harmonic imaging in the anesthetized dog Vol. 1.10 pp. 67-82

RDR-98-17: Lack of hemolytic potential of ultrasound energy after intravenous administration of DMP115 in the anesthetized dog. Vol.1.10 pp.119-130

Walker K. W., Pantely, G.A., & Sahn, D. (1997): Ultrasound-mediated destruction of contrast agent. Investigative Radiology, Vol. 32, 10, 728-734

Unger E. et.al.: Liposomes as myocardial perfusion ultrasound contrast agent: In Ultrasound-Contrast agents. B. Goldberg (editor, pps.177-178)

Evaluation of MRX 115: an ultrasound contrast agent: A summary of efficacy and Toxicity. Vol.1.10. pps. 180-206 Study was performed at

Effects of MRX 115 on monitoring placental flow in gravid baboons. The study was performed at Vol. 1.10 pp. 220-227

Myocardial perfusion characteristics and hemodynamic profile of MRX-115, a venous echocardiographic contrast agent during acute myocardial infarction. Vol.1.10. pp. 239-340.

Study #2286: General Pharmacology study of YM454 (DMP-115).
conducted the study. Study was in compliance with Good
Laboratory Practice Regulation. Vol. 1.10. pp. 357-403

RDR 98-12: Pharmacokinetics, distribution, metabolism and excretion of ¹⁴C-DMP 115 following an intravenous dose to conscious Sprague Dawley rats. Study was conducted at DuPont Pharmaceuticals. Vol. 1.11 pp.61-88

T98-06-02: An acute intravenous injection toxicity study in Cynomolgus monkeys followed by a 14-day recovery period. In-Life 06/23/98-07/0798.
Lot no 4509Z. GLP study. Vol.1.15 pp.214

T97-09-15: DMP115: 28-day intravenous dose toxicity study in Sprague-Dawley rats. DuPont Pharmaceutical Company, Newark, DE. Non-GLP. In-Life not provided. Lot No. PP974-024. Vol. 1.19 pp. 1-58

T97-11-05: DMP 115: a 28-day intravenous injection toxicity study in Sprague- Dawley rats.
GLP study. In-Life 11/11/97-12/12/97. Lot No.
PP974-024. Vol. 1.19 pp.59-326 and Vol. 1.20 pp.1-294

T-98-3-46: DMP 115: Acute/28-Day intravenous injection toxicity study in Sprague-Dawley rats followed by a 4-week recovery period. GLP study. In-Life 4/6/98-6/2/98 Lot No. 4508Z, 7474-71-0004. Vol. 1.21 pp.1-387 and Vol.1.22 pp.1-353

T98-5-2: DMP-115: A 28-Day intravenous injection toxicity study in Cynomolgus monkeys followed by a 28-Day recovery period. GLP study. In-Life 6/16/98-88/12/98. Lot No. 4509Z. Vol. 1.26 pp.1-271 and Vol. 1.27 pp.1-314

T98-07-02: Salmonella-Escherichia Coli/ Mammalian-Microsome reverse mutation assay preincubation method with a confirmatory assay. GLP study. In-Life: 7/24/98-8/18/98. Lot No: PP97A-024. Vol. 1.31 pp.1-44

T98-07-03: DMP 115: Chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory trial. GLP study. In-Life: 07/24/98-09/17/98. Lot No: PP97A-024. Vol. 1.31 pp.115-160

T98-07-04: DMP 115: DMP 115: In vivo rat micronucleus assay. GLP study. In-Life: 07/10/98-08/4/98. Lot No: PP97A-024. Vol. 1.31 pp.181-214

T97-09-52: Intravascular irritation study of MRX-115 in rabbits. GLP study. In life: 12/03/96-01/28/97. Lot No:744-71-0004. Vol. 1.31 pp.261-273

T97-09-53: Intramuscular irritation study of MRX-115 injection (Aerosomes) in rabbits. GLP study. In life: 1/20/97-2/3/97. Lot No:744-71-0004. Vol. 1.31 pp.274-290

T97-11-35: Eye irritation test of MRX 115 injection in rabbits. GLP study. Lot No: 744-71-0004. Vol. 1.31 pp.411-423

T98-04-02: In vitro hemolysis tests of MRX-115 injection (Aerosomes) in human blood. GLP study. Lot No: 744-71-0004. Vol. 1.31 pp.424-432

RDR-98-05: Assessment of intra-arterial administration of DMP115 in the anesthetized rat Vol. 1.10 pp. 114-118.

T98-10-29: Dose-response of DMP 115 and perfluoropropane in conscious rats following an intravenous dose. Vol. 1.31 pps: 402-410

Studies Submitted to the IND and previously reviewed by

Dr Dundore:

Myocardial imaging with a new transpulmonary lipid-fluorocarbon echo contrast agent: Experimental study in pigs. The study was carried out at Oregon Health Science Center by Dr. S. Grauer and published in American Heart Journal, (1996) Vol.132: 938-945

MRX115, an echocardiographic contrast agent, produces myocardial opacification after intravenous injection in primates: Studies before and after occlusion of left anterior descending

coronary artery. The study was conducted center by
Vol. 1.10 pp. 217-219

EB-ASO 10-94 and EB-ASO-11-94: Hematological and hemodynamic effects in rabbits induced by intravenous injection of MRX115, an ultrasound contrast agent from ImaRx 9EB-ASO 10-94 and EB-ASO 11-94. The study was conducted at Vol.1.10. pp. 228-243

SC950020: Cardiovascular evaluation of liposome encapsulated gas in Cynomolgus monkeys. Study was conducted by , in compliance with US Good Laboratory Practice Regulations. Vol. 1.10 pp.245-317

T95-07-25: Single dose IV toxicity study in rats with Aerosomes. Study CHV 2725-101. In-Life 7/20/94- 8/11/94. Lot no. PD071294. GLP study. Vol.1.13 pp. 384

T95-07-26: Intravenous tolerance study in Beagle dogs with Aerosomes. Study CHV 2725-100. In-Life 5/19/94-7/5/94. Lot no. KTW06074. Non-GLP study Vol. 1.14 pp.1-55

T95-07-27: Single dose toxicity study in beagle dogs with Aerosomes™. Study CHV 2725-102. In-Life 8/31/94-9/16/94. Lot no. PD071294. GLP study. Vol. 1.14 pp. 56-251

T95-07-29: 7-Day intravenous toxicity study in rats with Aerosomes™. In-Life 07/29/94-09/27/94. Lot No: KTW071294 GLP study. Vol. 1.16 pp.1-266

T95-07-22: 28-Day intravenous toxicity study of Aerosomes™, with a recovery period in rats. In-Life 07/29/94-12/27/94. Lot No: KTW102894. GLP study. Vol. 1.17 pp.1-322 and Vol. 1.18 pp. 1-304

T95-07-28: 7-Day intravenous toxicity study in dogs with Aerosomes™. In-Life 07/29/94-10/7/94. Lot No: KTW102894. GLP study. Vol. 1.23 pp. 1-266

T95-06-34: One-week intravenous toxicity study in the dog. Non GLP study. Lot No: PPD07-1294. Vol. 1.24 pp.1-76

T95-08-42: 7-Day repeated toxicity study in Beagle dogs with MRX-115. In-Life 01/9/95-03/15/95. Lot No: 744-71-0001. GLP study. Vol. 1.24 pp. 77-251

T95-97-24: ~~28-Day~~ intravenous toxicity study in cynomolgus monkeys with MRX-115 In-Life 12/06/94-1/05/95. Lot No: 744-71-0001. GLP study. Vol.1.25 pp. 1-335

T95-07-21: DMP 115: Mutagenicity test on MRX 115 in the L5178^{TK+} mouse lymphoma forward mutation assay with a confirmatory assay. GLP study. In-Life: 12/14/94-2/1/95. Lot No: 744-71-0001. Vol. 1.31 pp.215-260

Dr Sadrieh:

MRI 4490-F: In-vivo kinetics of the perfluoropropane component of MRX-115 in the dog. The study was conducted at Vol.1.11 pp.6-60

MR1 4312: ¹⁴C-PEG 5000 Products: Pharmacokinetics and disposition following intravenous administration to the rats. Vol.1.11. pp. 212-329.

MRI 4743: The effect of processing temperature on blood perfluoropropane levels. This study was carried out at GLP study. Lot No. 744-71-004

T97-09-51: Single intravenous dose toxicity study of MRX-115 in Cynomolgus monkeys. Study no; 396215. In-Life 12/10/96-12/25/96 Lot no: 744-71-0004 GLP study. Vol. 1.14 pp.252-293

T95-08-40: Dose range-finding developmental toxicity study in rats with MRX 115. GLP study. In-Life 3/27/95-4/19/95. Lot No: 744-71-0001. Vol. 1.28 pp. 230-349

T96-02-24: Rat developmental toxicity study with MRX-115. GLP study. In-Life 3/27/95-4/19/95. Lot No: 744-71-0001. Vol.1.29 pp.1-285

T97-02-54: Study of fertility and early embryonic development to implantation in rats with MRX 115. GLP study. In-Life 4/4/96-7/31/96. Lot No: 744-71-0002. Vol.1.28 pp.228

T95-08-41: Dose range-finding developmental toxicity study in rabbits with MRX 115. GLP study. In-Life 4/10/95-5/1/95. Lot No: 744-71-0001. Vol. 1.29 pp. 289-377

T95-02-25: Rabbit developmental toxicity study with MRX 115. GLP study. In-Life 4/10/95-5/1/95. Lot No: 744-71-0001. Vol.1-30 pp.1-237

T97-02-53: Antigenicity study of MRX-115 in guinea pigs. GLP study. Lot No: 744-71-0004. Vol. 1.31 pp. 291-345

T96-10-31: Antigenicity study of MRX-115 in guinea pigs. GLP study. Lot No: 744-71-0004. Vol. 1.31 pp. 346-401

Studies not reviewed within this submission:

MRI 4497:

RDR 98-13:

RDR 98-18:

RDR 98-29:

RDR 98-14:

RDR 98-30:

Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. Circ, 1987; 97:473-483 Vol.1.10 pp. 341-353

T95-7-23:

PHARMACOLOGY STUDIES:

RDR 98-26: Correlation of DMP 115 image intensity with bubble concentration Vol. 1.9 pp. 236-250 DuPont Pharmaceuticals report dated 10/1/98 Lot #4505 Z (final formulation). GLP: No

Design: The study was designed to evaluate the relationship between bubble concentration and ultrasound signal intensity in vitro. A custom made clinical ultrasound system was used for imaging (.). Imaging was performed using both fundamental and second harmonic gray scale modes in the absence, or presence of various concentrations of DMP-115 (0.08 - 1.6 μ L/L) diluted in 0.25% bovine serum albumin. Net video intensity values were calculated by subtracting the background values from the average raw video intensity value. Particle sizing was done using an Accusizer model 770A.

Results: For both fundamental and second harmonic modes of image acquisition, changes in bubble concentration were directly correlated with signal intensity. The video intensity of the second harmonic signal was significantly greater than the intensity of the fundamental signal. According to the sponsor, this resulted from changing the ultrasound system gain to take advantage of the additional dynamic range for the contrast signal in second harmonic mode.

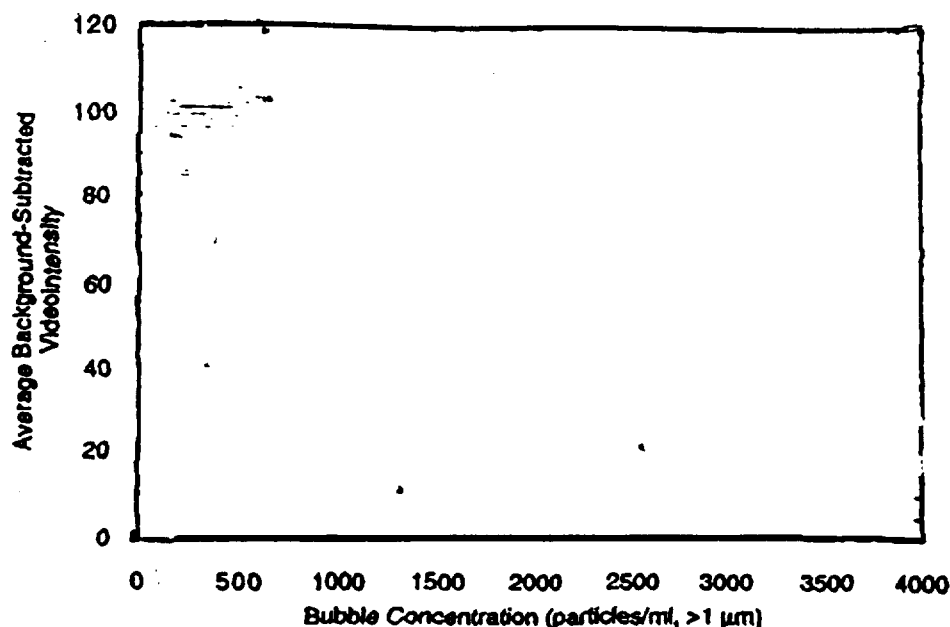


Figure 1: Video intensity versus bubble concentration for fundamental and second harmonic imaging modes. The result of the logarithmic curve fit is shown. The values for both modes decrease monotonically with decreasing bubble concentration and go to zero when no agent is present. The r-value were 0.984 and 0.978 for the fundamental and second harmonic studies respectively.

Sponsor's Conclusions: The sponsor concluded that ultrasound video intensity is directly related to the concentration of bubbles from DMP 115

Reviewer's comments: Agree with the study conclusion about the in vitro relationship between bubble size and signal intensity.

RDR 98-20: Determination of the optimal dose of DMP 115 for assessment of ventricular opacification and second harmonic imaging in the anesthetized dog Vol. 1.9 pp. 292-307 DuPont Pharmaceuticals report dated 9/1/98 (lot. # 744-71-0004) GLP: No

Design: The study examined the optimal dose of intravenously administered DMP 115 needed for fundamental left ventricular opacification and gated second harmonic myocardial perfusion in open-chest pentobarbital anesthetized dogs. Male and female dogs (N=4) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate and indices of myocardial contractility. DMP115 was prepared using a Vialmix™ set for 45 seconds of agitation after which it was allowed to stand for 5 minutes at room temperature before infusion intravenously at 3, 10 and 30 μl/kg (X 0.08, 0.27 and 0.81 MHD based on body surface area (MHDbsa)) over two minutes.

Results: Dose-related increases in the duration of left ventricular opacification and in the video intensity duration of myocardial perfusion of the left circumflex and left anterior descending coronary artery were obtained. Myocardial opacification lasted for between 9 – 14 minutes. No significant difference in video peak intensity was observed because of intense ventricular

shadowing occurring at 30 $\mu\text{L/kg}$. DMP 115 at the doses evaluated did not alter mean arterial pressure, heart rate or myocardial contractility.

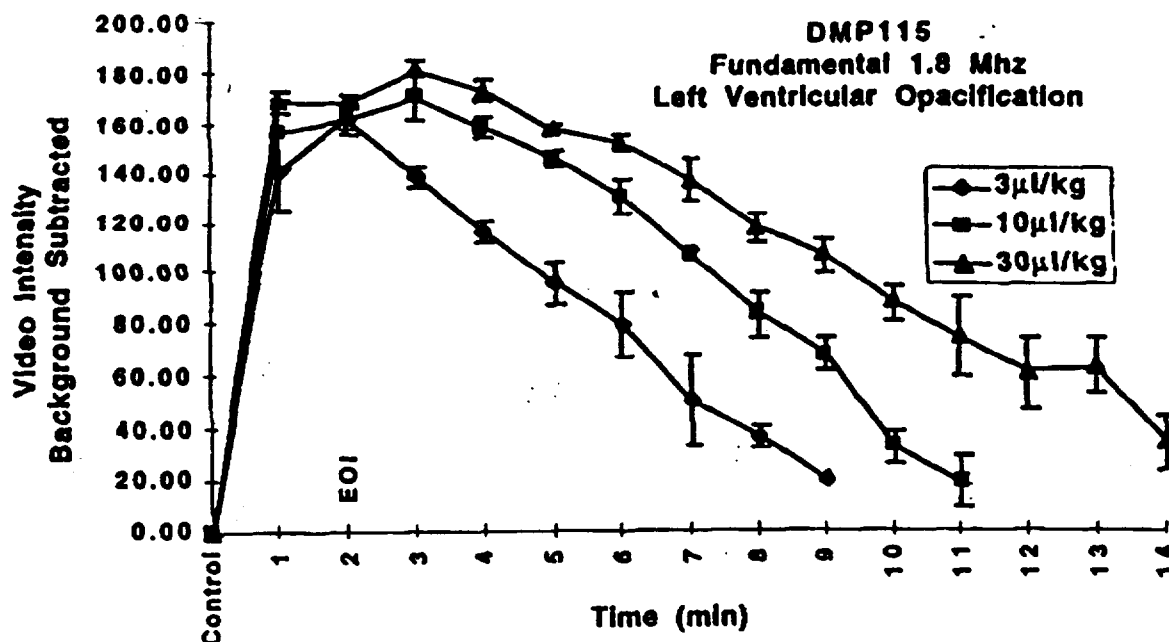


Fig 1: Time-intensity curve for DMP 115 fundamental left ventricular opacification. Each line is the mean \pm SEM; N=4 EOI= end of infusion

Table 4. Area Under the Curve, Peak Video Intensity, Time to Peak Response and Duration of Useable Imaging for DMP115 fundamental left ventricular opacification.

Parameters ^a	3 $\mu\text{L/kg}$	10 $\mu\text{L/kg}$	30 $\mu\text{L/kg}$
AUC (VI \cdot min)	954 \pm 38	1394 \pm 30 ^b	1661 \pm 109 ^{bc}
Peak VI	172 \pm 8	181 \pm 5	184 \pm 7
Duration ^d (min)	8.5 \pm 0.5	10.5 \pm 0.3	13.5 \pm 0.5
Time to Peak (min)	1.75 \pm 0.25	2 \pm 0.6	2.5 \pm 0.5

a. All values are expressed as the mean \pm SEM; N=4
AUC = Area Under the Curve, VI = Video Intensity

b. Significant difference from 3 $\mu\text{L/kg}$ at $P \leq 0.05$ using Newman-Keuls test.

c. Significant difference from 10 $\mu\text{L/kg}$ at $P \leq 0.05$ using Newman-Keuls test.

d. Duration of useable imaging time was defined as time during which VI > 25 VI units

Conclusions: The sponsor concluded that a dose of 10 $\mu\text{L/kg}$ DMP 115 infused for two minutes produced the optimal dose for demonstrating left ventricular opacification and myocardial perfusion.

Reviewer's comments: I agree with the overall conclusion that the doses used produced the required echocardiography. However, it does not appear that the 10 $\mu\text{L/kg}$ dose was necessarily superior to the 3 $\mu\text{L/kg}$ dose. For both doses, peak image intensity was similar, although the signal duration was about 2 minutes longer for the 10 $\mu\text{L/kg}$ dose. Given that this slight advantage in duration was achieved by increasing the dose about 3 fold, I would have settled for the lower dose. Although monitored, the ECG results were not provided, the reason for the omission was not stated.

RDR98-21: Assessment of DMP 115 4 days post-preparation on ventricular opacification and second harmonic imaging in the anesthetized dog Vol.1.10 pp. 2-23 DuPont Pharmaceuticals report dated 9/10/98 (lot. # 4505Z, final formulation) GLP: No

Design: The study design was similar to study RDR 98-20 (previous study) with the exception that DMP 115 was infused 5 minutes or 4 days post- Vialmix™ preparation. Male and female dogs (N=4) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate and indices of myocardial contractility. DMP115 was prepared using a Vialmix™ set for 45 seconds of agitation after which it was allowed to stand for 5 minutes at room temperature before infusion intravenously at 3, 10 and 30 $\mu\text{L/kg}$ (X 0.08, 0.27 and 0.83 MHD based on body surface area (MHDbsa)) over two minutes. Before the infusion, the vials containing DMP 115 were hand inverted ten times. Particle sizing data were acquired with Accusizer model 770A.

Result: Dose-related increases in the duration of left ventricular opacification and in the video intensity duration of myocardial perfusion of the left circumflex and left anterior descending coronary artery were obtained. A dose of 10 $\mu\text{L/kg}$ resulted in optimal, homogenous opacification of the left ventricle without ventricular shadowing. No significant difference

Table 3. Summary of the number of particles/ml for 5 minutes and 4 days post preparation as determined by an Accusizer.

Parameters ^a	Number of Particles/ml 5 min. post preparation	Number of Particles/ml 4 days post preparation
1 - 2 μm	35E+08 \pm 2.5E+08	9.2E+08 \pm 0.5E+08
2 - 6 μm	3.0E+08 \pm 0.2E+08	1.5E+08 \pm 0.1E+08
6 - 10 μm	5.6E+07 \pm 0.4E+07	4.4E+07 \pm 0.6E+07
> 10 μm	1.6E+07 \pm 0.2E+07	0.82E+07 \pm 0.03E+07

a. All values are expressed as the mean concentration \pm SEM; N=11 for 5 minutes and N=12 for 4 days post preparation.

attributable to amount of time elapsing between preparation of DMP 115 and its utilization was evident.

**DMP115 4 days vs. 5 min. post vialmixing
Left Ventricular Opacification (1.8MHz)**

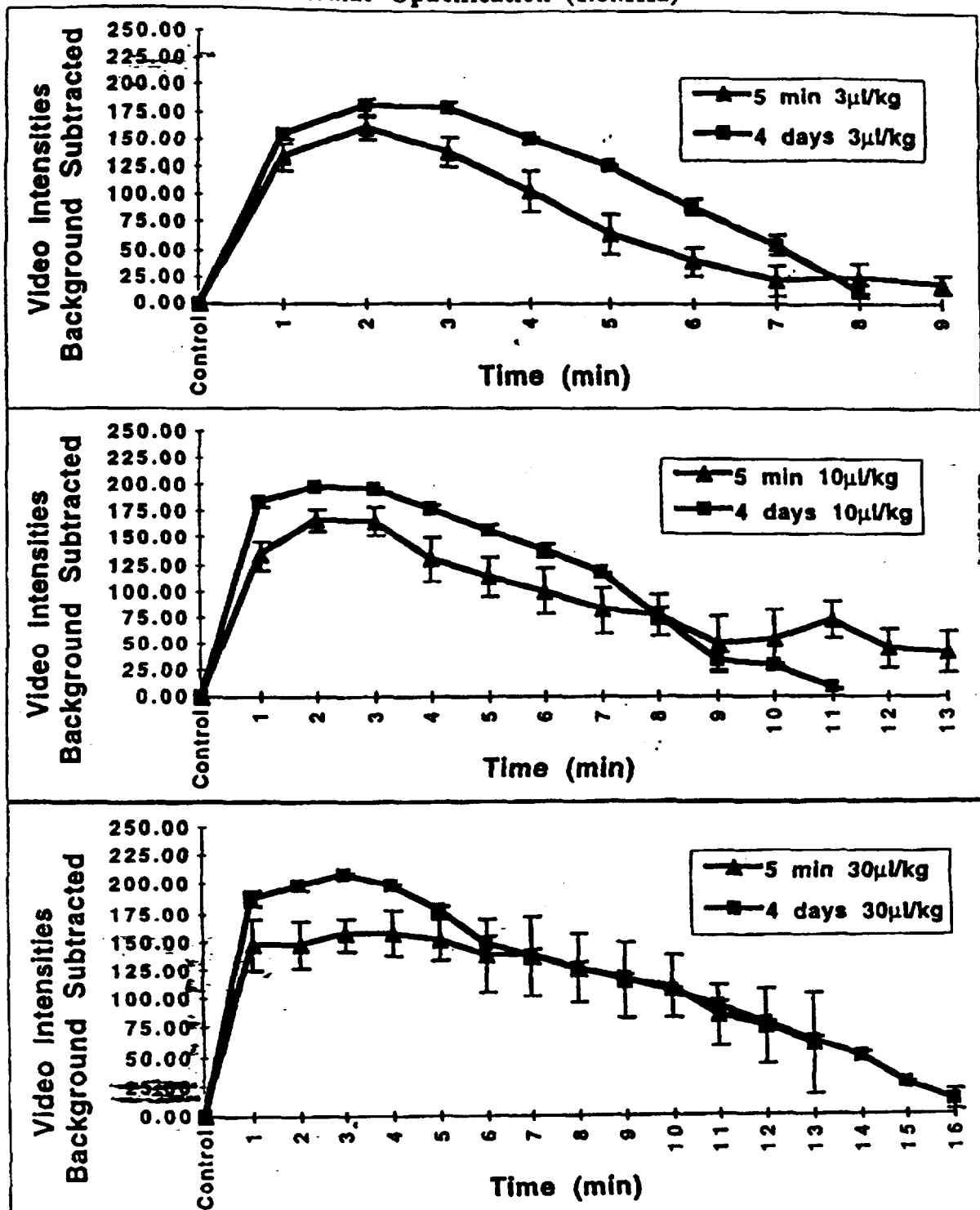


Figure 5. Depicted above are typical background subtracted left ventricular time/intensity curves for DMP115 4 days and 5 minutes post preparation. The top graph represents a dose of 3 µl/kg while the middle 10 µl/kg and the bottom graph 30 µl/kg IV in the canine 2 minute infusion protocol. Each line is the mean \pm SEM; N=4 except 30µl/kg 5 minutes where N=3.

Conclusions: The sponsor concluded that DMP 115 should be efficacious and not pose a risk to patients who are administered this agent up to four days post preparation.

Reviewer's comments: I agree with the sponsor that in this study, DMP 115 remains efficacious in producing echocardiographic demonstration of the left ventricle and of myocardial perfusion up to four days post preparation. The video intensity appears even better for the four day post preparation sample despite the fact that number of particles per ml remaining was less after four days. However, since the toxicological, immunological and other indices of safety were not examined, the sponsor's conclusion that the said preparation does not pose a risk to patients is not supported by the data. Interestingly, the number of particles decreased after four days, yet video intensity remained the same, suggestive that a lower dose of DMP 115 might be sufficient to obtain clinical efficacy. This point was also raised in my comments for study RDR 98-20

RDR 98-15: Comparison of stability of DMP 115 ultrasound signal in saline or canine whole blood using clinically relevant power levels of ultrasound. Study was conducted by DuPont Pharmaceuticals, Billerica, MA dated 10/1/98 pp. 214-235 GLP: No

Design: The study assessed the behavior of DMP 115 in canine whole blood or in saline using clinically relevant power level of ultrasound. The experiments were performed using a clinical ultrasound system at two different power levels. DMP 115 was diluted to a concentration of 1:750 or 1:800 in either blood or saline and exposed to ultrasound in one of two small test cells. Two series of experiments were performed. In the first series, the imaging performance of DMP 115 was assessed in blood and saline at room temperature and atmospheric pressure. In the second series, the response of DMP 115 was examined at 37°C with, and without pressurization. The images were recorded and digitized. The mean video intensity for a region within the cell as a function of time was calculated and plotted. The average plots and the mean integrated video-intensity (area under the normalized time-video intensity curve) were compared for both saline and blood.

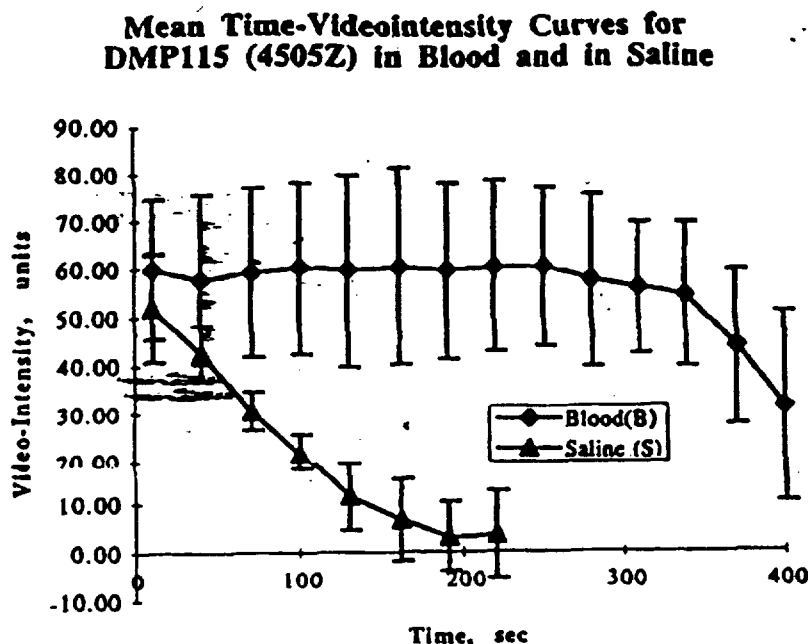


Fig1: Time-videointensity-plot for non-pressurized experiments in blood and saline at mechanical index of 0.7 (n=8).

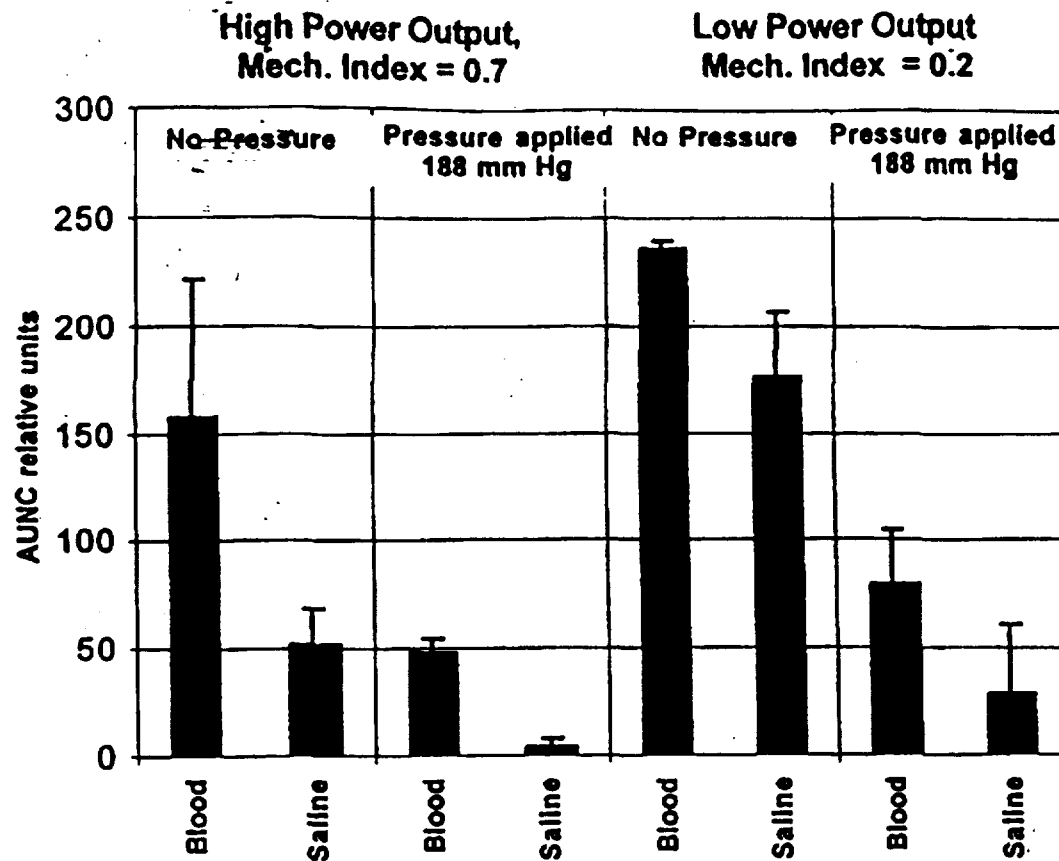


Fig. 2: AUNC (Area-under-normalized-curve) data for the portion of the study of the study using pressurized cells. According to the sponsor, it is evident from this that the bubbles survive substantially longer in blood than in saline even when stressed by high ultrasound pressure and high ambient pressure. It can also be seen that effect of these two stressors is synergistic; increased acoustic power decreases survival time even further when pressure is applied than it does at atmospheric pressure

Results & Conclusions: In both cases, the duration of action was significantly greater for the dilution in blood. For a mechanical index (MI) of 0.7 (at 1.67 MHz) the $t_{1/2}$ for signal decay was 400 seconds in blood versus 90 seconds in saline. The mean integral of the normalized video intensity over time was in all cases greater for the dilution in blood than those in saline. Low concentrations of bovine serum albumin added to saline substantially prolong the contrast effect. For both power levels and both suspending media, increasing the pressure decreases bubble life. The sponsor concluded that DMP 115 is more stable to ultrasound exposure when diluted in blood as compared to when diluted in saline. Furthermore, the rate of disappearance of contrast agent in blood as well as in saline is related to the acoustic output of the ultrasound scanhead and to increases in the ambient pressure.

Reviewer's comments: Although the sponsor did not give any definition of clinical relevant power level of ultrasound, I agree with the study conclusions that DMP 115 appears more stable to ultrasound exposure when diluted in blood as compared to when diluted in saline. Furthermore, that the rate of disappearance of contrast agent in blood as well as in saline is related to the acoustic output of the ultrasound scanhead and to increases in the ambient pressure.

RDR 98-16: An in vitro assessment of DMP 115 fragility under exposure to ultrasound at different power levels. Study was conducted by DuPont Pharmaceuticals, Bilerica, MA. Study date: 10/1/98. Vol.1.9 pp. 251-273. GLP: No

Study summary:

The study was performed to investigate the behavior of DMP 115 (lots 4500 and 4505Z) under both acoustic fields of varying intensity and combinations of varying acoustic fields and elevated static pressure. Measurements were made of acoustic attenuation at 2.25 MHz over a fixed path-length of approximately six centimeters in ml of air-saturated physiological saline at 37°C using a range of static head pressures and low acoustic intensities. Rate constants for the decrease of attenuation with time were compared for increasing levels of acoustic pressure in a low range and showed a direct relationship between the two that could fit using a second-order polynomial. At the lowest intensity, the rate was indistinguishable from baseline with a value of 0.009 dB/ minute. No significant difference was found in either initial attenuation or rate of attenuation decrease between the two lots.

RDR 98-22: Comparison of DMP 115 manufactured via an process for assessment of ventricular opacification and second harmonic imaging in the anesthetized dog. Vol.4.10 pp. 24-46 DuPont Pharmaceuticals report dated 9/10/98 (lot. # 4505Z, final formulation or Lot # GMP4)

Design: The study examined whether differences in the manufacturing process for DMP 115 impacted on its ability to produce ventricular opacification and second harmonic imaging in the anesthetized dog. To produce the lipid blend in the formulation process, for the three lipids were mixed in water followed by For the three lipids were dissolved in a mixture of and After concentration, was added and the solids isolated by followed by Open-chest dogs (# of animals for each study stated in the result section) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate and indices of myocardial contractility. DMP115 was prepared using a Vialmix™ set for 45 seconds of agitation after which it was allowed to stand for 5 minutes at room temperature before infusion intravenously over one minute at 3, 10 and 30 µl/kg (X .08, 0.27, 0.81 MHDbsa).

Results & conclusions: Both methods of preparing lipid blend produced comparable opacification of the left ventricle and perfusion of the coronary arteries.

The duration of usable imaging time was similar. There was no significant effect on hemodynamic or hematological parameters. The sponsor concluded that intravenous infusion of DMP 115 produced by in open-chest canine opacified the left ventricle and perfused the myocardium similarly.

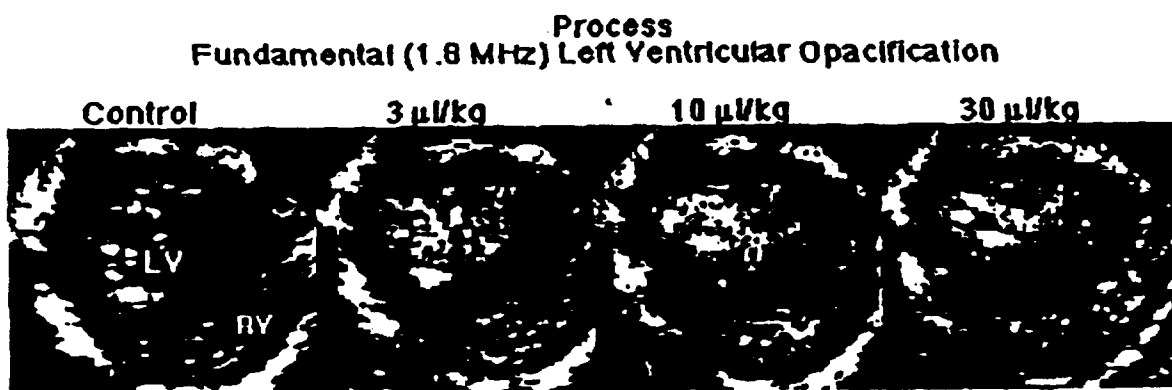
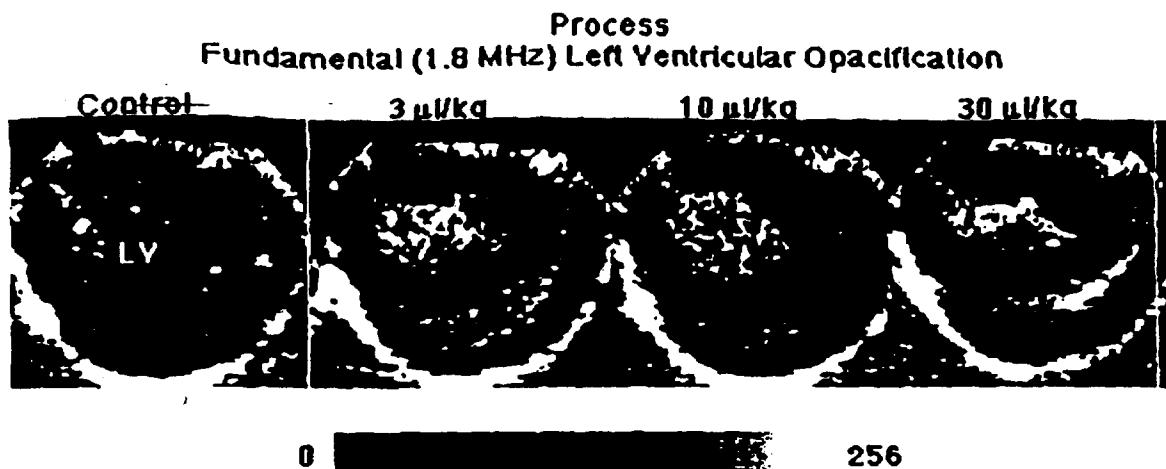


Figure 6. Depicted above are typical fundamental (1.8Mhz) echocardiographic short axis images of the heart. The upper panel represents left ventricular opacification with DMP115 process while the bottom panel shows DMP115 process at increasing doses. LV=left ventricle, RV=right ventricle.

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DMP 115
Left Ventricular Opacification (1.8 MHz)

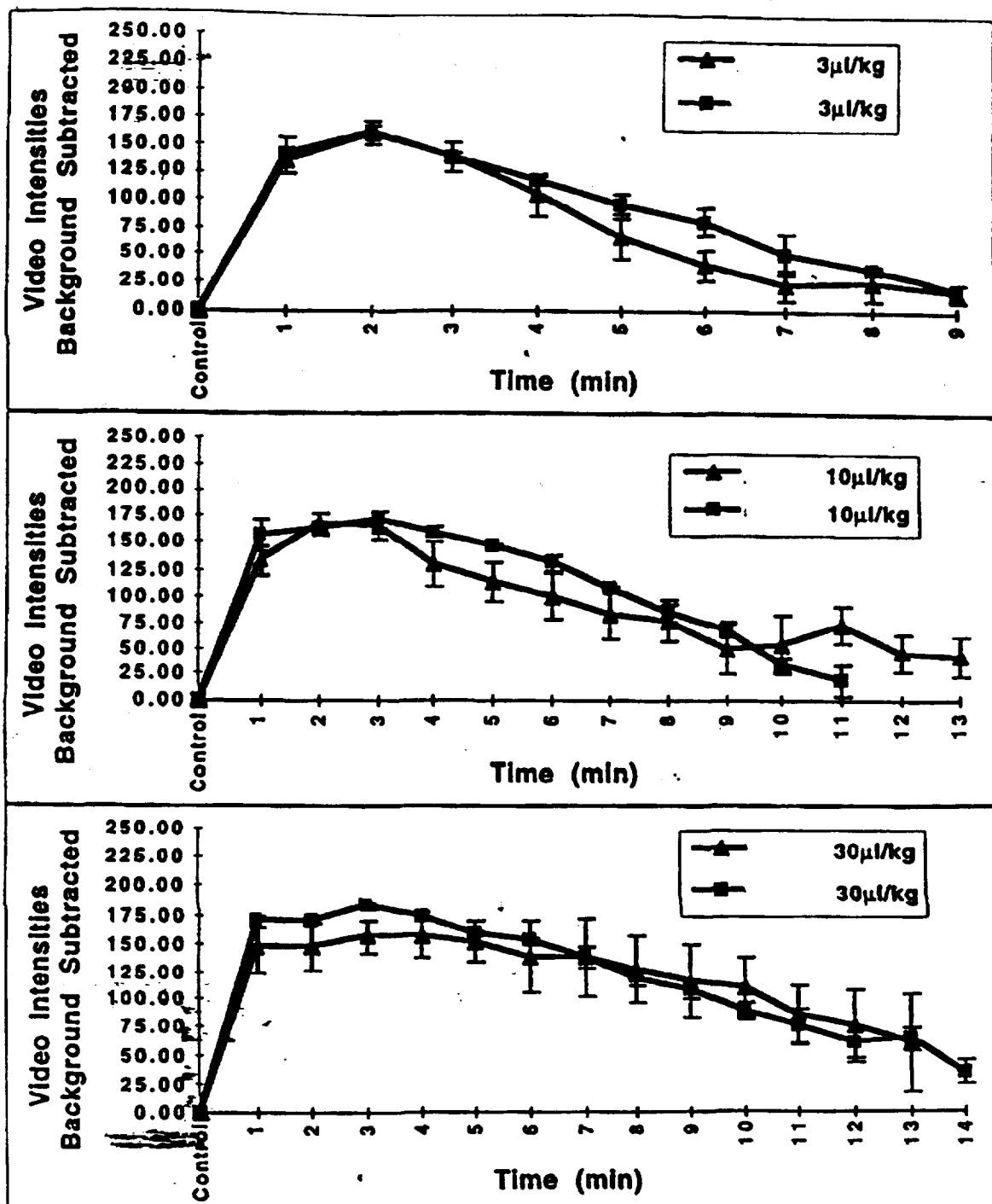


Figure 5. Depicted above are typical background subtracted left ventricular time/intensity curves for DMP 115. The top graph represents a dose of 3 μ l/kg while the middle 10 μ l/kg and the bottom graph 30 μ l/kg IV in the canine 2 minute infusion protocol. Each line is the mean \pm SEM; N=4 except 30 μ l/kg where N=3.

Reviewer's comments: Agree with the conclusions that the method of preparation does not affect imaging outcome.

RDR98-23: Dose optimization and comparison of various modes of administration on ventricular opacification and second harmonic imaging in the anesthetized dog Vol.1.10 pp.47-66 DuPont Pharmaceuticals report dated 09/16/98. GLP: No

Design: The study examined the influence of three methods of administering DMP 115 on the magnitude and duration of fundamental left ventricular opacification and gated second harmonic myocardial perfusion. The methods of administration were; 1) As a bolus over 10 sec; 2) as infusion over two minutes and, 3) diluted into a 50 ml I.V. saline bag and infused at a rate of 9 μ l/kg for a 30 minutes period. Doses ranging from 0.3-30 μ l/kg were examined.

Open-chest pentobarbital anesthetized adult beagle dogs (# of animals for each study stated in the result section) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate and indices of myocardial contractility. DMP115 was prepared using a Vialmix™ set for 45 seconds of agitation after which it was allowed to stand for 5 minutes at room temperature before administration.

Results and conclusions: Both the bolus and 2 minute infusion method produced dose-related increases in image intensity and duration of action (area under the curve). Bolus injection resulted in left ventricular AUCs of 175 ± 22 , 238 ± 56 and 1632 ± 190 VI•min for doses of 0.3, 1, 3 and 10 μ l, respectively. DMP 115 infused over 2 minutes resulted in left ventricular AUCs of 954 ± 38 , 1394 ± 30 and 1661 ± 109 VI•min for 3, 10 and 30 μ l /kg, respectively. With the bolus injection ventricular shadowing occurred at 10 μ l/kg compared with 30 μ l/kg for the 2 minutes infusion. DMP 115 infused over 30 minutes resulted in steady state left ventricular opacification without ventricular shadowing, with AUC of 77 ± 10 occurring from 1 to 30 minutes. However, the peak video intensity and the AUC values were less when compared with bolus or 2 minutes infusion. Myocardial perfusion results were qualitatively similar to those described for left ventricular imaging. DMP115 infused over 2 minutes resulted in a dose-related increase in VI of 97 ± 3 , 121 ± 2 , and 136 ± 2 for doses of 3, 10 and 30 μ l /kg for left circumflex coronary artery respectively. The duration of gated harmonic myocardial perfusion (GHMP) were 5.1 ± 0.6 , 6.4 ± 0.4 , and 9.9 ± 0.9 respectively occurring at the end of infusion. The sponsor stated that DMP 115 did not alter hematological or hemodynamic parameters studied although these values were recorded only at the end of the infusion period.

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ON ORIGINAL

DMP115 Fundamental Left Ventricular Opacification

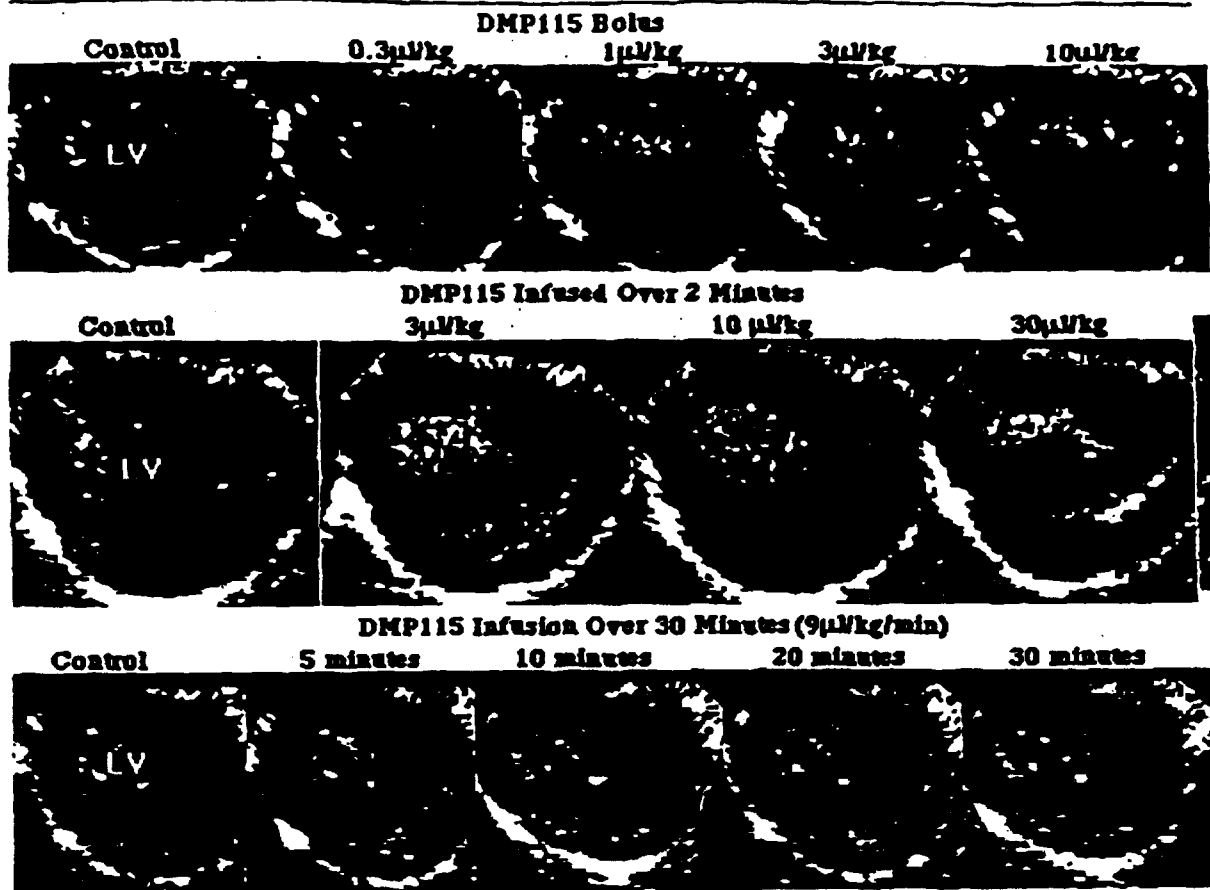


Figure 3. Depicted above are typical left ventricular opacification images of the heart post DMP115 administration. The upper panel represents DMP115 administered as increasing bolus doses while the middle DMP115 administered over 2 minutes and the bottom panel DMP115 administered over 30 minutes. LV = Left Ventricle.

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**Fundamental 1.8 MHz
Left Ventricular Opacification**

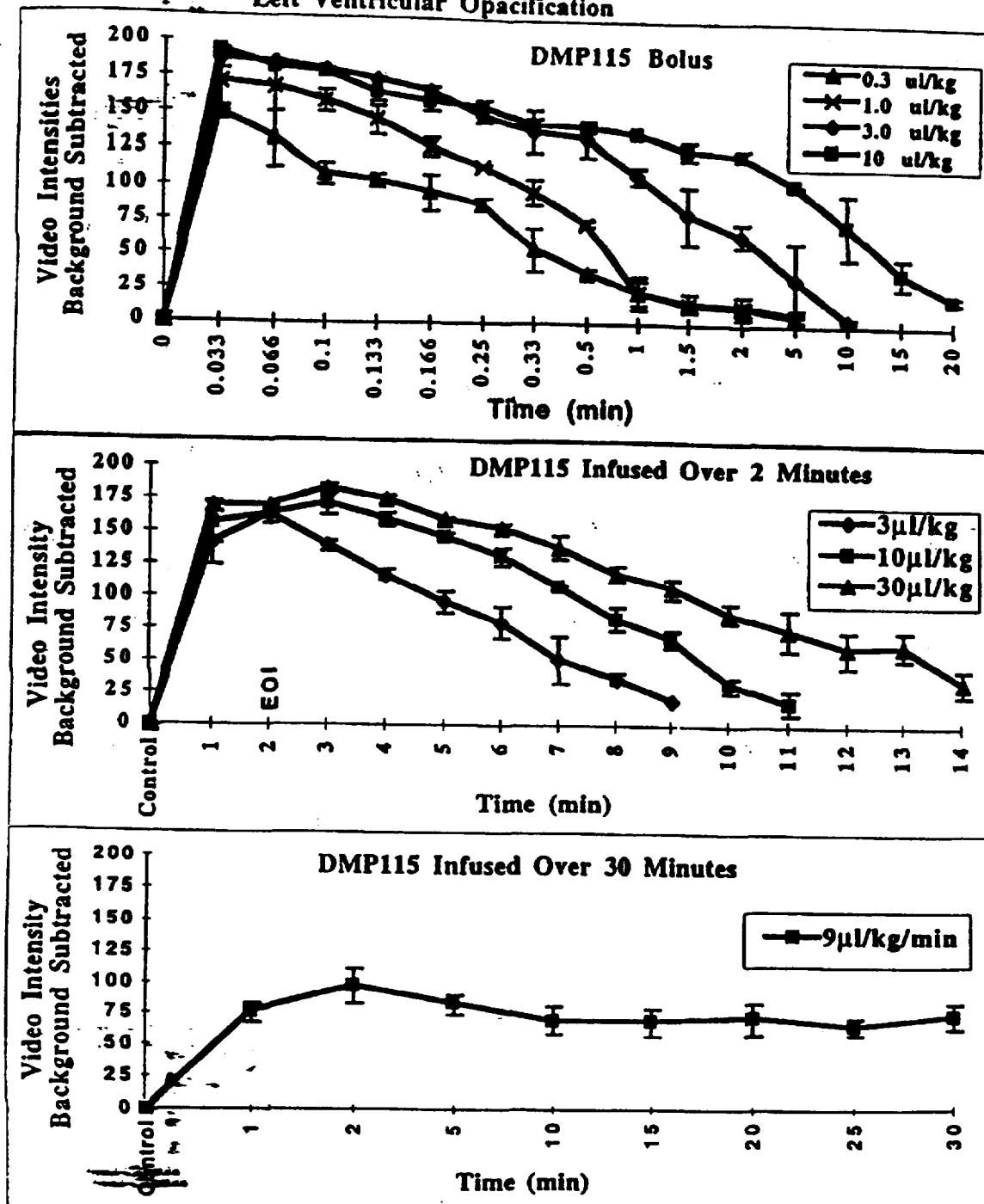


Figure 2. Depicted above are typical background subtracted left ventricular time/intensity curves for DMP115. The top graph represents bolus doses of DMP115 while the middle 2 minute infusions and the bottom a 30 minute infusion of DMP115. Each line is the mean \pm SEM; N=4 except DMP115 Bolus where N=2.

Reviewer's comments: Agree with the conclusion about imaging intensity and duration. The sponsor stated that hemodynamic and hematological parameters were recorded only at the end of the infusion, making it impossible to assess the changes that occur during infusion.

RDR-98-19: Determination of the ability of DMP 115 to detect myocardial perfusion defects in a canine model of occlusion/ reperfusion using gated second harmonic imaging. Vol. 1.10 pp. 84-96. DuPont Pharmaceutical report dated 03/09/98 Lot 744-71-0004. GLP: No

Design: The study examined the ability of intravenously infused DMP 115 to detect myocardial perfusion defects in the canine left circumflex (LCX) occlusion/reperfusion model.

A segment of the LCX was isolated and fitted with a vascular occluder. DMP 115 (10 µl/kg) was infused I.V. over two minutes at the following time points: control, 20 and 60 minutes of occlusion, during reactive hyperemia, and 1 and 2 hours post reperfusion. Images were recorded using a Sonos 2500 with second harmonic capability (1.8/3.6 MHz) gated at every 10th end systolic and diastolic beat. Upon completion of the experiment, the heart was excised and stained for the determination of risk areas and infarct size.

Results: There was homogeneous, end-systolic myocardial perfusion with DMP 115 during control infusion, with LCX video intensities (VI) of 148±6 and 154±9 compared with background (no contrast) VIs of 40±6 and 45±3 for LCX and LAD areas respectively. Perfusion defects determined by lack of contrast within the LCX were visible with mean background subtracted VI's of 23±6 and 14±6 as compared to 97±6 and 98±7 within the LAD during the occlusion period at 20 and 60 minutes, respectively. Similarity existed between pathological staining and occlusion images along with a no reflow phenomenon occurring during reactive hyperemia.

Conclusion: The sponsor concluded that DMP 115 perfused the myocardium and accurately detected myocardial perfusion defects in the anesthetized canine using gated second harmonic imaging.

Reviewer's comments: Agree

RDR 98-27: Assessment of DMP 115 responses to pressure and ultrasound in normal saline at 37°C. A comparison of six qualification lots manufactured at two sites. Vol.1.9 pp.170-179. DuPont Pharmaceutical Company report dated 10/1/98 GLP: No

Design: The study compares six final formulation lots prepared using the same method but manufactured at two different sites in terms of acoustic attenuation, rate of decay of attenuation and response of the attenuation to increasing ambient pressure in saline at 37°C. DMP 115 samples prepared by agitation in VialmixTM for 45 seconds and allowed to stand for five minutes were subjected to low intensity ultrasound in a closed, stirred 10 ml test cell filled with air saturated saline at 37°C. Measurements were made of the intensity of the ultrasound frequency spectrum that was reflected from a stainless steel block prior to, and after the addition of the test agent and the values obtained converted to dB. For the pressure assays, the cells were subjected to a stepwise increasing ambient pressure every 20 seconds in 10 steps of 10 mmHg from atmospheric to 100 mmHg. Particle sizing data were obtained with an Accusizer model 770A.

Results: There was no significant difference among the six lots studied in terms of acoustic attenuation or response to pressure.

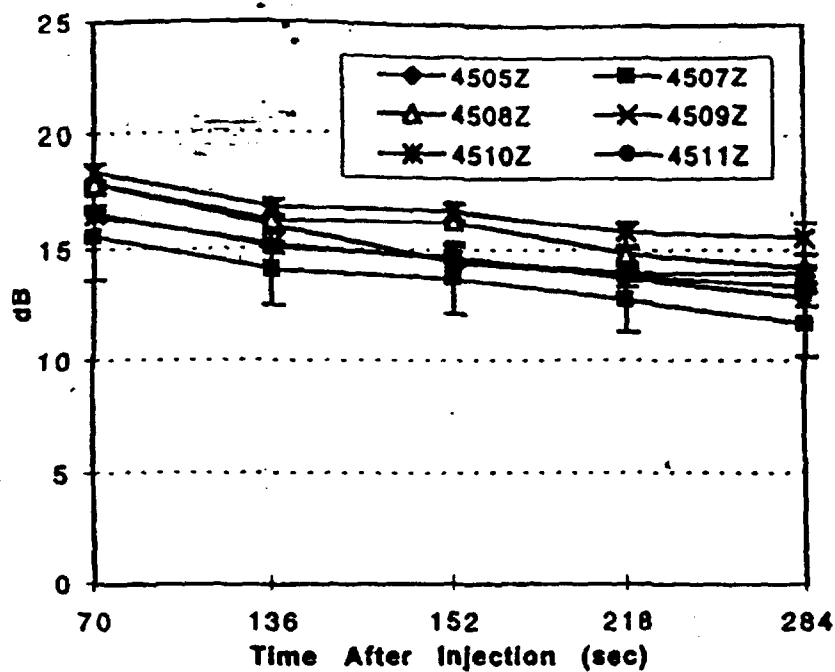


Figure 1: Attenuation at 2025 MHz as a function of time for DMP 115 in saline at 37°C. Data is expressed as the mean \pm the standard deviation for each lot. There is no significant difference among the lots studied.

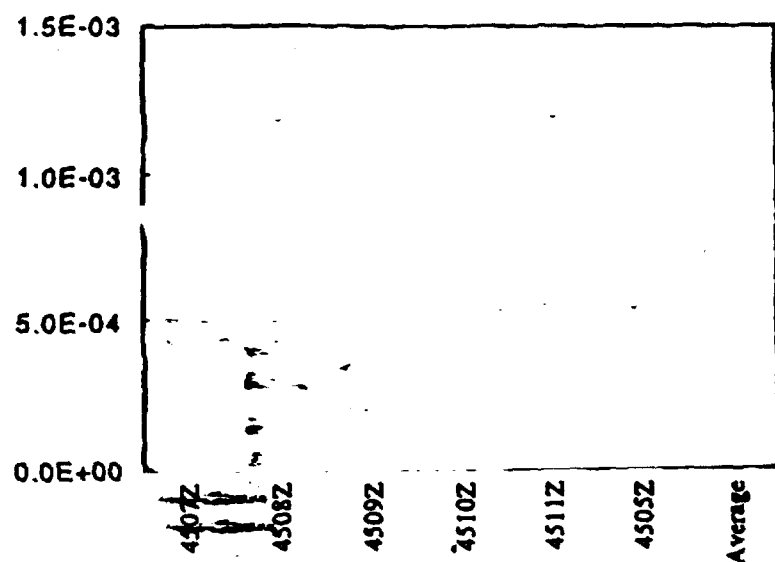


Figure 3: Rate of decrease of attenuation for DMP 115 in saline at 37°C. One way analysis of variance indicates that there is no significant difference among the six lots.

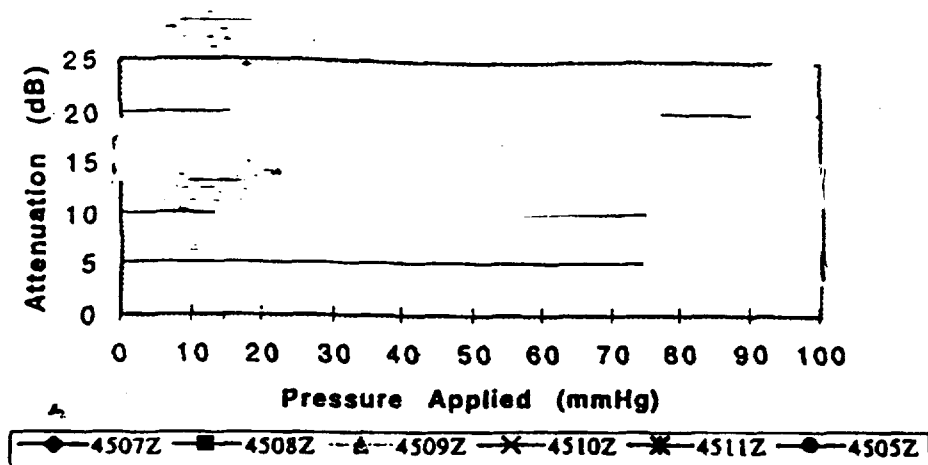


Figure 4: Acoustic attenuation of DMP 115 versus static pressure in 37°C saline. There is no significant difference among any of the lots tested.

Conclusion: There was no significant difference among the six lots studied in terms of acoustic attenuation or response to pressure.

Reviewer's comments: Agree with the conclusion.

RDR98-24: Determination of effect of DMP 115 bubble size on ventricular opacification and second harmonic imaging in the anesthetized dog Vol. 1.10 pp. 67-82. DuPont Pharmaceutical Company report dated 9/10/98. GLP: No

Design: The study examined the effect of bubble size on fundamental, (1.8 MHz) and second harmonic, (1.8/3.6 MHz) imaging. Open-chest pentobarbital anesthetized adult beagle dogs of either sex (# of animals for each study stated in the result section) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate, ECG, and indices of myocardial contractility. For particle size preparation, post agitation samples were pooled together and fractionated into a top and bottom portion via buoyancy in a 60 ml separating funnel. Each fraction was then analyzed via an Accusizer™. The top fraction contained a significantly larger number of particles/ml between 2-10 μm than the bottom fraction; $150 \times 10^6 \pm 16 \times 10^6$ vs $3.6 \times 10^6 \pm 0.8 \times 10^6$. The number of particles/ml in the 1-2 μm range were similar in both top; $6.2 \times 10^8 \pm 0.4 \times 10^8$ and bottom fraction; $5.0 \times 10^8 \pm 0.8 \times 10^8$. Both fractions were infused over two minutes intravenously at increasing doses of 0.003, 0.01, 0.3 and 1 $\mu\text{l/kg}$. Fundamental imaging of the left ventricle (LV) was recorded over six minutes. A second study was also performed assessing the effect of continuous second harmonic imaging with the same dose for each fraction.

Results: There were dose-related increases in video intensity for both fractions under fundamental and second harmonics. Top and bottom fractions at 0.003 $\mu\text{l/kg}$ did not elicit quantifiable backscatter using fundamental or second harmonic ventricular imaging. Doses of 0.01, 0.3 and 1 $\mu\text{l/kg}$ resulted in a significantly number of particles > 2 μm for the bottom fraction as compared to the top fraction, while particles between 1-2 μm remained similar. Fundamental left ventricular peak video intensities for the top fraction were significantly higher than the bottom fraction at doses of 0.01 and 0.3 $\mu\text{l/kg}$. Top and bottom fractions demonstrated no difference in video intensity at 1 $\mu\text{l/kg}$. Results using second harmonic imaging demonstrated no effect at 0.003 and 0.01 $\mu\text{l/kg}$. At doses of 0.3 and 1.0 $\mu\text{l/kg}$, the bottom fraction contained a

significantly smaller number of particles between 2-10 μm but elicited equivalent second harmonic video intensities as the top fraction.

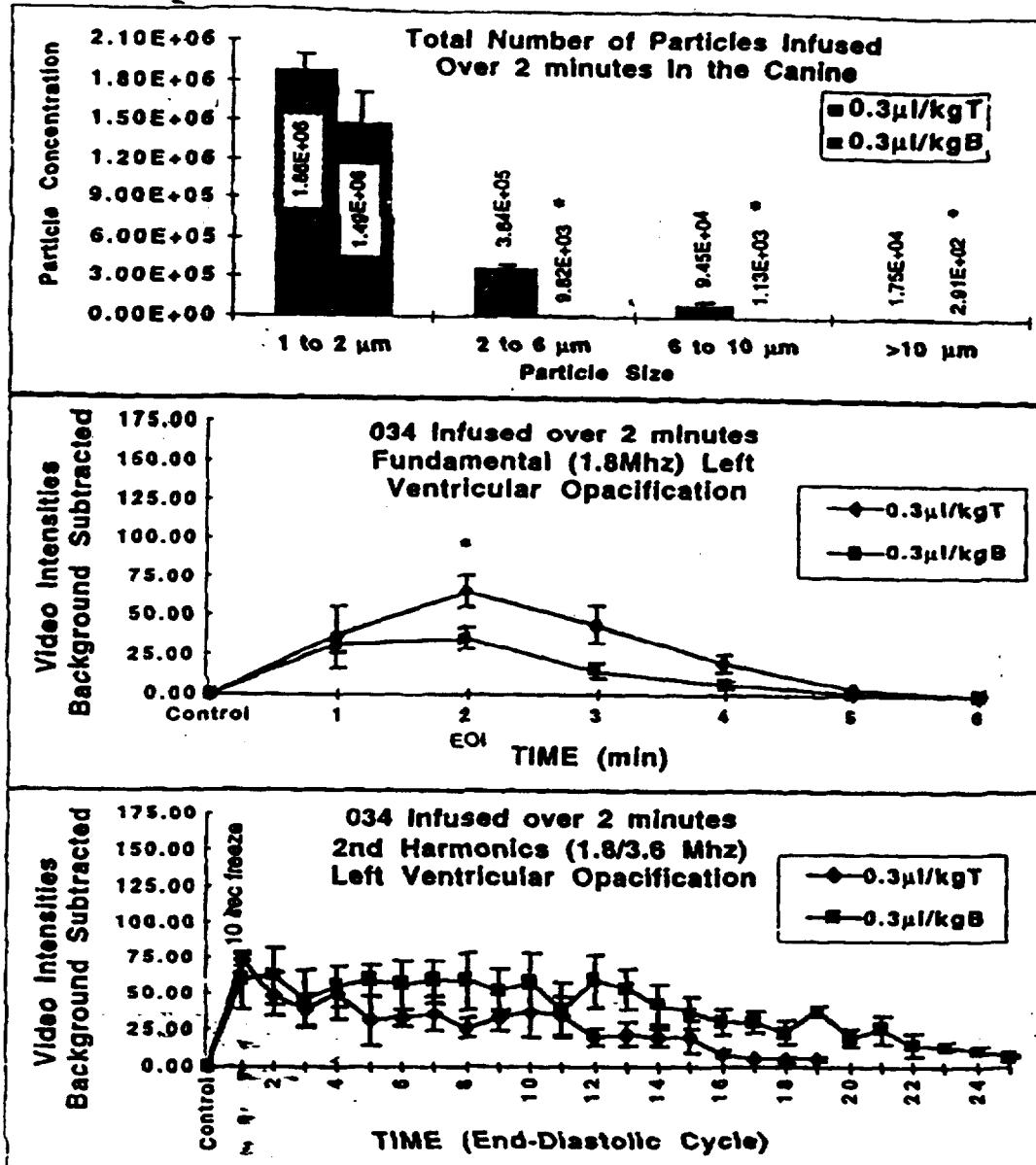


Figure 5. Depicted is the particle size analysis for a dose of 0.3 $\mu\text{l/kg}$ and corresponding background subtracted left ventricular video intensities for fundamental and 2nd harmonics. Shown in the top graph is the distribution and number of particles infused in the canine over 2 minutes. The middle graph depicts the left ventricular video intensities for fundamental (1.8 MHz) imaging while the bottom graph shows left ventricular video intensities for continuous 2nd harmonics (1.8/3.6MHz) corresponding to the dose. Each histogram or line is the mean \pm sem for 4 canines. * Indicates significant difference from the top fraction using Student's unpaired t-test ($P \leq 0.05$). T = Top Fraction, B = Bottom Fraction and EOI = End Of Infusion

Conclusion: The sponsor concluded that DMP 115 bubbles between 2-10 μm are the primary contributors for imaging using the fundamental mode while the 1-2 μm bubbles are the primary contributors for imaging in the second harmonic mode.

PHARMACOLOGY SUMMARY

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed as a contrast-enhancing agent for clinical ultrasound. The sponsor stated that the submitted data support the use of Definity™ for contrast-enhanced echocardiographic imaging of cardiac structure.

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator.

The sponsor submitted the results of several studies examining the ability of Definity™ to produce contrast enhancement in vitro, and in a number of animal species. Study RDR 98-26 was an in vitro study designed to evaluate the relationship between bubble concentration and ultrasound signal intensity in vitro. The results demonstrated that changes in bubble concentration were directly correlated with signal intensity for both fundamental and second harmonic modes of image acquisition. Moreover, the video intensity of the second harmonic signal was significantly greater than the intensity of the fundamental signal. The sponsor posited that the enhanced signal resulted from changing the ultrasound gain to take advantage of the additional dynamic range for the contrast signal in the second harmonic. RDR 98-15 examined the stability of DMP 115 ultrasound signal in saline and canine blood. Two series of experiments were performed. In the first, the imaging performance of DMP 115 was assessed in blood and saline at room temperature and atmospheric pressure. In the second series, the response was examined at 37°C both with and without pressurization. In both cases, the duration of signal generated was significantly greater for the dilution in blood. For both media, increasing the pressure decreased microbubble life.

Efficacy studies were performed in dogs (studies RDRs 98-19, 98-20, 98-21, 98-22, 98-23, pigs (American Heart Journal, Vol. 1996, 132 938-945), and monkeys. In general the animals were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate, ECG, and indices of myocardial contractility. The results showed that Definity™ produced dose-related increases in the duration of left ventricular opacification and in the video intensity duration of myocardial perfusion of the coronary arteries. In these species, the highest dose examined was generally limited by concomitant increase in ventricular shadowing reducing the resultant peak image intensity. RDR 98-23 was a dose optimization study and comparative analysis of three methods of DMP-115 administration on the magnitude and duration of fundamental left ventricular opacification and gated second harmonic myocardial perfusion in open-chested dog. The methods of administration were: 1) as a bolus over 10 sec; 2) as infusion over two minutes and, 3) diluted into a 50 ml intravenous saline bag and infused at a rate of 9 µl/kg for a 30 minutes period. Both the bolus and the 2 minutes infusion method produced dose-dependent increases in image and duration of action. Ventricular shadowing occurred at a lower dose with bolus injection compared with infusion over 2 minutes. DMP-115 infused over ~~30-35~~ minutes resulted in steady state left ventricular opacification without ventricular shadowing but peak intensity was about half that of bolus injection.

RDR-98-24 examined the effect of bubble size on fundamental and second harmonic imaging in anesthetized dogs. For particle size preparation, post agitation samples were pooled together and fractionated into a top and bottom portion via buoyancy in a 60 ml separating funnel with the top fraction containing a significantly larger number of particles/ml between 2-10 µm than the bottom fraction; $150 \times 10^6 \pm 16 \times 10^6$ vs $3.6 \times 10^6 \pm 0.8 \times 10^6$. The number of particles/ml in the 1-2µm range were similar in both top; $6.2 \times 10^8 \pm 0.4 \times 10^8$ and bottom fractions; $5.0 \times 10^8 \pm 0.8 \times 10^8$. Both fractions were infuse intravenously over two minutes at increasing doses of

0.003, 0.01, 0.3 and 1 $\mu\text{L/kg}$. Fundamental imaging of the left ventricle (LV) was recorded over six minutes. A second study was also performed assessing the effect of continuous second harmonic imaging with the same dose for each fraction. There were dose-related increases in video intensity for both fractions under fundamental and second harmonics. Doses of 0.01, 0.3 and 1 $\mu\text{L/kg}$ resulted in a significantly smaller number of particles $> 2\mu\text{m}$ for the bottom fraction as compared to the top fraction while particles between 1-2 μm remained similar. Fundamental left ventricular peak video intensities for the top fraction were significantly higher than the bottom fraction at doses of 0.01 and 0.3 $\mu\text{L/kg}$. Top and bottom fractions demonstrated no difference in video intensity at 1 $\mu\text{L/kg}$. Results using second harmonic demonstrated no effect at 0.003 and 0.01 $\mu\text{L/kg}$. At doses of 0.3 and 1.0 $\mu\text{L/kg}$, the bottom fraction contained a significantly smaller number of particles between 2-10 μm but elicited equivalent second harmonic video intensities as the top fraction. The sponsor concluded that DMP 115 bubbles between 2-10 μm are the primary contributors for imaging using the fundamental mode while the 1-2 μm bubbles are the primary contributors for imaging in the second harmonic mode.

RDR 98-21 evaluated the effect of time of preparation on the effectiveness of DMP-115. DMP-115 was evaluated five minutes- or 4 days-post preparation. No significant difference in video intensity characteristics attributable to amount of time elapsing between preparation of DMP-115 and its utilization was evident.

Manufacturing process for the lipid component of the drug substance was changed during development from an _____ to an _____

RDR 98-22 examined whether the differences impacted on the ability of DMP-115 to produce required echocardiography. The results showed that both methods of preparing lipid blend produced comparable opacification of the left ventricle and perfusion of the coronary arteries. RDR 98-27 compares six formulation lots manufactured at two different sites in terms of acoustic attenuation, rate of decay of attenuation and response of the attenuation to increasing ambient pressure in saline at 37°C. There was no significant difference in terms of acoustic attenuation or response to pressure among the lots studied.

SAFETY PHARMACOLOGY:

RDR 98-17: Lack of hemolytic potential of ultrasound energy after intravenous administration of DMP 115 in the anesthetized dog VOL.1.10 pp.119-130. DuPont Pharmaceutical Company report dated 9/10/98. GLP: No

Design: The study examined the potential of DMP115 to elicit hemolysis. The postulate was that in vitro ~~energy~~ release from ultrasonographic cavitation processes will result in RBC membrane destruction.

Open-chest pentobarbital anesthetized adult beagle dogs (n=3) were prepared for echocardiography probe, measurement of mean arterial pressure, heart rate and indices of myocardial contractility. Whole blood was collected via catheters placed in the left ventricle and femoral artery. Platelet, WBC and RBC counts, hematocrit and hemoglobin determinations were performed on whole blood and serum. Base line data were collected prior to infusion of DMP 115 prepared using a Vialmix™ set for 45 seconds of agitation after which it was allowed to stand for 5 minutes at room temperature before administration. Doses administered were 10

$\mu\text{l/kg/2 min}$ and $40\text{ }\mu\text{l/kg/2min}$ (X0.27 and 1.1 MHDbsa respectively). Imaging was done using a 1.6/3.34 MHz echocardiographic probe.

Results and conclusions: One animal has plasma hemoglobin level of 0.025g/dl. Hemoglobin was not detected in the plasma of the remaining 3 dogs. Serum from blood lysed with Sigma lysing solution gave a serum hemoglobin concentration of 3.3g/dl. The sponsor described the change in the affected dog as minimal. Whole blood CBC was not significantly altered by mechanical index, imaging paradigm or by the presence of DMP115. The sponsor concluded that DMP115 in the presence of high mechanical index and at clinically relevant doses did not induce hemolysis.

Reviewer's comments: The sponsor should have evaluated the effect of higher dose multiples, nevertheless, at the doses examined, I agree with the conclusion that DMP115 does not induce hemolysis at the dose examined.

Evaluation of MRX 115 an ultrasound contrast agent: A summary of efficacy and Toxicity.
Vol.1.10. pp. 180-206. Study was performed at
Study dated 04/19/94. GLP: No

Design: The study evaluated the safety and efficacy of intravenously administered MRX 115 $1\text{--}10\text{ }\mu\text{l/kg}$ (0.026-0.27 MHDbsa) in 3 anesthetized dogs instrumented for echocardiography probe, measurements of arterial pressure, heart rate and pulmonary arterial pressure. Color Doppler images were collected from peripheral organs using power Doppler imaging techniques. Non-cardiac images were obtained using an ultrasound system. The study also determined the acute intravenous toxicity of MRX 115 in mice.

Results: MRX 115 at all doses examined produced myocardial enhancement that lasted for 30-90 sec with attenuation of cardiac images occurring at $10\text{ }\mu\text{l/kg}$ dose level. The sponsor stated that echogenicity material remained in the left ventricular cavity for extended periods of time (20 minutes). Color Doppler signal enhancement was noted in the liver, testes and kidney. No changes in systemic blood pressure, pulmonary arterial blood pressure or heart rate were observed. Post mortem examination showed slight congestion of the posterior dorsal lobe of lungs.

For the acute intravenous toxicity study in mice, the LD_{50} was approximately 8.9 ml/kg (X36 MHDbsa). Signs of toxicity included dyspnea, tachypnea, ataxia, hyporeactivity, hyperreactivity, convulsion and death.

Conclusions: MRX 115 administration produced ventricular opacification and increased the power of the color Doppler signals from the testes, liver and kidneys. The LD_{50} in mice was 8.9 ml/kg .

Reviewer's comments: Agree with the study conclusions.

Myocardial imaging with a new transpulmonary lipid-fluorocarbon echo contrast agent: experimental study in pigs. The study was carried out at Oregon Health Science Center by Dr. S. Grauer, and published in American Heart Journal, Vol.132: 938-945 GLP: No

Dr. Dundore reviewed this study for the IND.

Study summary.

Anesthetized pigs (n=7) received MRX-115 at 0.5, 1, 2, 5 and at 10 $\mu\text{L/kg}$ (X 0.025-0.5 MHDbsa) i.v. in random order. Transthoracic echocardiography showed dose-dependent myocardial opacification of both ventricular cavities. Peak videodensity lasting up to 5 minutes occurred at 5-10 $\mu\text{L/kg}$. There was no change in heart rate, systolic blood pressure, or pO_2 . However, 5 and 10 $\mu\text{L/kg}$ of MRX-115 increased mean pulmonary pressure (PAP) transiently by 13 and 16 mm Hg respectively. The PAP returned to baseline within 8 minutes after dosing. The authors ascribed the elevation of PAP to thromboxane-mediated pulmonary hypertension caused by pulmonary intravascular macrophage response to injected particles.

Reviewer's comments: The authors suggested that the elevation in PAP might be susceptible to inhibition by cyclooxygenase inhibitors such as aspirin or indomethacin. Such a study was not conducted. This reviewer opined that DuPont should have investigated the mechanisms involved in the elevation of PAP further. The investigation is pertinent in the light of reports by the medical reviewer that some of the subjects developed significant hypotensive response that necessitated their removal from the study. The lack of an effect on the pO_2 was not surprising since pO_2 was analyzed 15 minutes after each injection, a time period that did not correspond to the time frame of the reversible pulmonary hypotension response. Therefore, the possibility that there was a transient change in pO_2 at the time of increase in PAP can not be excluded.

MRX115, an echocardiographic contrast agent, produces myocardial opacification after intravenous injection in primates: Studies before and after occlusion of left anterior descending coronary artery. The study was conducted by Dr. Grauer and colleagues at Oregon Health Sciences and published in Acta Radiol (1996) 3: S405-S406. Vol. 1.10 pp. 217-219. GLP: No

Dr. Dundore reviewed this study for the IND.

Study summary.

Two procedures were utilized for echocardiographic evaluation in Rhesus monkeys: transthoracic (T), followed in the same animals by open chest epicardial (OCE) echocardiography before and after occlusion of the left anterior descending coronary artery. 0.5-100 $\mu\text{L/kg}$ (X 0.008-1.62 MHDbsa) were evaluated for T while for OCE, 20 μL or 50 $\mu\text{L/kg}$ were examined. Myocardial opacification was quantifiable at all doses examined with the 100 $\mu\text{L/kg}$ producing significant shadowing of the image. MRX-115 at all doses studied, did not affect heart rate, pulmonary arterial pressure, systemic arterial pressure or pO_2 (data for the hemodynamic changes were not provided for evaluation).

Reviewer's comments: The results obtained in open-chest epicardial echocardiography study demonstrated delineated filling defect that corresponded to the area of compromised wall motion. The filling defect became reversed upon reperfusion.

Effects of MRX115 on monitoring placental flow in gravid baboons. The study was performed at
1.10 pp. 220-227. GLP: No Vol.

The study was performed on four gravid baboons (gestational age \approx 150 days) instrumented to measure systemic arterial pressure in the thoracic aorta and pulmonary artery. All the animals were sedated. Two were allowed to breathe without assistance while the remaining animals were paralyzed (agent not provided), intubated and ventilated. Two dimensional ultrasound images of the placental using color Doppler and gray scale methodologies were recorded. A bolus intravenous dose of 100 μ l/kg MRX-115 (X2.70 MHDbsa) used for imaging revealed blood flow through the arcuate artery and intervillous space. In the preliminary experiments, other contrast agents examined were Echovist (i.a.), Filmix (i.v.) and agitated saline (i.v.). The doses employed were not provided. The reporting laboratory stated that "of the four contrast agents administered, Echovist administered intra-arterially demonstrated superior efficacy since it gave the greatest signal intensity compared to the other agents". One baboon had a stillborn. According to the sponsor, "further investigation concluded that it was not due to the treatment with the study drug" Details of the investigation were not provided. Moreover, the particular animal was said to have had a history of a stillborn fetus on two other occasions and during the study was under anesthesia for an extended period of time and slow to recover (no reason was given for this animal longer duration under anesthesia).

Reviewer's comments: The study did not provide sufficient details for evaluation. It is particularly unfortunate that no detail of the pathophysiological findings on the stillborn fetus was provided in view of the results of the reproductive toxicology studies

EB-ASO 10-94 and EB-ASO 11-94: Hematological and hemodynamic effects in rabbits induced by intravenous injection of MRX115, an ultrasound contrast agent from ImaRx. The study was conducted at
Vol. 1.10. pp. 228-243. GLP: No

Dr. Dundore reviewed this study for the IND.

Study summary.

The hematological study was conducted in 9 conscious male Chinchilla rabbits. Six of the animals received a single i.v. bolus injection of 75 μ l/kg MRX-115. Remaining 3 were injected with saline. Arterial blood samples were collected for hematological analysis, prior to and up to 60 minutes after administration of MRX-115. There was no effect on blood platelets, total white blood cells, granulocytes or lymphocytes.

The hemodynamic study was conducted in 3 pentobarbital-anesthetized, artificially ventilated Chinchilla rabbits prepared for measurements of systemic arterial and pulmonary pressure. 5, 15, 150 and 225 μ l/kg of MRX (0.073-5.6 MHDbsa) was administered intravenously in ascending order. Arterial blood samples were taken before, and at 10, 20, 30 and 60 minutes post injection for measurement of blood gases. The results showed that intravenous administration of MRX-115 produced no changes in systemic or pulmonary arterial pressures at doses up to 225 μ l/kg. The pH, pO_2 and pCO_2 of arterial blood were also unaffected.

Reviewer's comments: The contract laboratory stated that MRX-115 was sent by the sponsor in a "ready to use formulation with the quality guaranteed for approximately 10 days". received the vials on April 15, 1994. The length of time spent in transit was not stated. The experiments were conducted from April 19-22, 1994. How this might have impacted the

formulation bubble size distribution and other physico-chemical properties were not commented on although the results of study RDR 98-21 showed that the bubbles are okay up to 4 days after mixing.

SC950020: Cardiovascular evaluation of liposome encapsulated gas in cynomolgus monkeys. Study conducted by Vol. 1.10 pp.245-31. In life phase
02/17/95-04/14/95. GLP: Yes

Dr. Dundore reviewed this study for the IND.

Study summary.

4 monkeys were surgically implanted with radiotelemetric blood pressure and EKG transmitters. Following a 10-day recovery period, animals were conditioned to 8-hour restraints. Baseline cardiovascular data were obtained for two days prior to treatment. All 4 animals were sequentially administered the following: 1) a saline control, 2) 50 μ /kg, 3) 100 μ /kg, and 4) 1.0 ml/kg of DMP-115 (X0.008- 16.2 MHDbsa) as bolus injections. A 2-hour wash out period was allowed between doses. Cardiovascular parameters were unremarkable in 3 of the 4 animals. A marked and physiologically significant fall in blood pressure and heart rate was noted in one of the 4 animals at 1 ml/kg. In this animal, the mean arterial pressure and heart rate were decreased by approximately 40 mm Hg (-38%) and 95 beats per minute (-46%). The decreases rebounded within 10 minutes and returned to near baseline in 45 minutes. Both parameters were not affected in the remaining animals. Continuous electrocardiographs were obtained from 24 hours before to 7 days after DMP-115 administration. The consultant Veterinary cardiologist report noted what was described as possible 2 ventricular premature depolarization originating from the specialized connective tissue of the left ventricle in one monkey and prolonged ST segments with alterations in the configurations of T waves that were consistent with electrolyte imbalance in another. He concluded that the findings did not reveal any abnormalities attributable to treatment.

Following a three-week washout period, the same animals in the above study were utilized for a study examining cardiovascular responses using dosage regimes comparable to what would be experienced under clinical stress echo conditions. 3 consecutive dosages of DMP-115 were acutely evaluated at 10 minute intervals. All 4 animals were administered 1 ml/kg saline followed at 10 minute intervals by 3 doses of 500 μ /kg DMP-115. EKG tracings were evaluated by a board certified veterinary cardiologist. No acute blood pressure or heart rate changes were noted. Additionally EKG evaluations were unremarkable for all animals.

Myocardial perfusion characteristics and hemodynamic profile of MRX-115, a venous echocardiographic contrast agent during acute myocardial infarction. Vol. 1.10. pp. 329-340. Dr. J. Linder conducted the study at the University of Virginia School of Medicine. The findings were published in J. Am. Soc. Echocardiography 1998, 11: 36-46

Design: The study examined the utility of MRX-115 to assess risk area and infarct size during coronary occlusion, and after reperfusion. Venous injection of 0.5 ml of MRX-115 (X 13.5 MHDbsa) was performed in 12 open-chest dogs during baseline, coronary occlusion and after reperfusion in the presence of exogenous hyperemia. Ultrasound was transmitted at 2 MHz and received at both fundamental and harmonic frequencies during continuous and intermittent imaging. The risk area during coronary occlusion was compared with technetium autoradiography, and the infarct size after reperfusion was compared postmortem utilizing tissue staining technique.

Results: Poor signal enhancement or posterior wall attenuation did not allow for the measurement of perfusion defect during continuous (both fundamental and harmonic) and intermittent (fundamental imaging). Measurement was possible during intermittent harmonic imaging. Correlation analysis between perfusion defect size on intermittent harmonic imaging and either autoradiographic risk area or postmortem infarct size gave r values of 0.83 and 0.92 respectively. There were no effects on hemodynamic or pulmonary gas exchange.

Conclusions: Dr. Linder concluded that MRX-115 accurately assessed regions of hypoperfusion when combined with intermittent harmonic imaging.

Reviewer's comments: agree.

Study No: 2286: General Pharmacology study of YM454 (DMP-115).
conducted study. Study was in compliance with
Good Laboratory Practice Regulation dated. Vol. 1.10. pp. 357-403

Design: The study evaluated the effect of DMP-115 on (1) hexobarbital sleeping time in mice and on body temperature in rats (2) Cardiovascular effect in anesthetized dogs (3) Urinary output and electrolyte balance in conscious rats. In all experiments, doses of DMP 115 utilized were 0.01, 0.1 or 1.0 ml/kg. For the hexobarbital sleeping time experiment, DMP 115 was administered intravenously followed fifteen minutes later by hexobarbital, 80 mg/kg intraperitoneally. Anesthesia time was measured, as the time required for the mice to change posture from a lateral position to a spontaneous prone position. Chlorpromazine 3mg/kg was used as positive control. There were 8 male mice in each group. For the body temperature experiments in rats, rectal temperature was measured using a thermistor before and at 0.25, .5, 1, 2, and 4 hours after the administration of DMP-115. Aminopyrine 200 mg/kg s.c was used as positive control.

Cardiovascular studies were carried out on four pentobarbital anesthetized dogs prepared surgically for measurement of mean arterial pressure, pulmonary arterial pressure, heart rate, indices of myocardial contractility, ECG, peripheral blood flow (femoral) and respiration. Arterial blood was collected to measure blood gases and pH. Measurements were made before administration and for the following periods after administration; vehicle 10 minutes, DMP 115 (0.01 and 0.1 ml/kg), 30 minutes; and DMP 115, 1.0 ml/kg, 60 minutes. Blood gases were measured at 10 minutes intervals before and after administration. For all studies, test substance was shaken in a special-purpose shaker and administered immediately.

Results: DMP-115 did not produce any change in hexobarbital sleeping time in mice whereas chlorpromazine 3 mg/kg prolonged sleeping time by 190% compared with the vehicle control. Effect on body temperature in rats was unremarkable. In addition, no significant change in urine volume, urinary excretion of Na^+ , K^+ or Cl^- , urinary Na^+/K^+ ratio or pH was produced by DMP-115 in rats.

For the cardiovascular study in dogs, DMP-115 at 0.01, 0.1, and 1 ml/kg produced no marked change in respiration, blood pressure, heart rate, femoral blood flow, left ventricular pressure, LV dp/dtmax, or electrocardiogram PP-R and Q-T intervals. Furthermore, no marked change in pO_2 , pCO_2 or HCO_3^- concentration in blood or pH was seen.

Conclusions: DMP-115 at doses up to 1 mg/kg, i.v. did not affect hexobarbital sleeping time in mice, body temperature, water or electrolyte balance in rats. There was no significant effect on cardiovascular parameters measured in dogs.

Reviewer's comments: Agree.

Published Literature submitted by the sponsor in support of the application:

In view of the fact that both the science and the regulatory issues associated with the use of microbubbles as ultrasound contrast agent are still evolving, I decided to provide a synopsis of literature materials submitted by the sponsor. It is hoped that the material will aid in our better understanding of the issues involved with the application.

Walker K. W., Pantely, G.A., & Sahn, D.: Ultrasound-mediated destruction of contrast agent. Investigative Radiology (1997), Vol. 32, 10, 728-734

This was a study conducted by Dr. Walker and his colleagues at the Division of cardiology, Medical University of South Carolina, Charleston. The study was performed to investigate how different parameters of ultrasound energy delivery influence the destruction of ultrasound contrast agents. The authors hypothesized that ultrasound intensity, duration and frequency were important determinants of ultrasound energy-mediated microbubble destruction. An in vitro system consisting of an elevated reservoir connected by thin-walled rubber tubing encased in agar was constructed. Flow was controlled at the outlet of the rubber tubing by a peristaltic pump. Samples were injected into the upstream section of tubing between the reservoir and the rubber tube. The agar served as a spacer and coupling between the transducer and the flow tube as well as a tissue phantom for system calibration. Bubbles were collected at the outflow and counted using an accuser. Ultrasound reflectivity was measured on-line with a Hewlett-Packard 2500 in both the standard (1.8/1.8 MHz) and harmonic modes (1.8/3.6MHz). Ultrasound energy power output was calculated assuming attenuation by the agar of 0.3dB/MHz/cm (a value assumed for human tissue). Contrast agents examined included MRX115 (DMP115), Imagent (AF0150), Levovist and Echogen

The authors noted that while no significant reduction in counts or reflectivity occurred at an ultrasound output of 0.3 W/cm², exposure to 25 W/cm² resulted in microbubble destruction, and reduction in reflectivity in all the contrast agents examined. Furthermore, the authors stated that declines in reflectivity were, increased by longer exposure to ultrasound, slower flow through the ultrasound beam, continuous rather than intermittent imaging, use of a higher pulse repetition rate, and exposure to higher frequency. While the authors noted the limitations of this in vitro system including the fact that the study was performed in water, not blood and the inherently non-linear relationship between microbubble concentration and reflected microbubble acoustic signal. Nevertheless, they concluded that optimization of ultrasound delivery system may be used to maximize or minimize the destruction of ultrasound contrast agent. Furthermore they raised the intriguing proposal that a potential application of ultrasound contrast agent would be the targeted delivery of therapeutic or diagnostic agent.

Reviewer's comments: Agree with the experiment-based conclusions of the study.

Unger E. et al.: Liposomes as myocardial perfusion ultrasound contrast agent: In Ultrasound-Contrast agents, B. Goldberg (editor, Mosby Press pp.57-74)

Dr. Evan Unger and colleagues began the book chapter with a historical review of contrast agents, the technology for detecting them and the potential role of myocardial perfusion imaging in medical practice. They continued with a discussion of lipid-encapsulated microbubbles as ultrasound contrast agents and the advantages of different lipids and lipid-states to limit gas permeability through the bubble shell. They went on to discuss the characteristics of MRX-115.

The size and surface appearance of MRX 115 was captured with optical photomicrographs and freeze fracture electron micrographs. The mean size distribution using Nycomps particles sizing system was 2.5µm. In contrast DuPont in-house studies suggested a mean diameter size of 1.5 µm. Low power in vitro acoustic measurements were made in isotonic saline. The attenuation values obtained were four times higher than those obtained in DuPont in-house studies. The authors did not mention the concentration of MRX-115 used for the study or the length over which attenuation was measured. The authors did not provide sufficient details to make for meaningful evaluation of the cardiovascular data.

SAFETY PHARMACOLOGY SUMMARY

The submission addressed the safety issues of Definity™ from many perspectives. R98-17 examined the potential of DMP 115 to induce hemolysis in dogs via in vivo energy release from ultrasonographic cavitation processes. The results showed lack of hemolysis.

Dr. Walker and his colleagues at the Medical University of South Carolina, in a paper reported in the Journal of Investigative Radiology (1997, Vol. 32 728-734) examined how ultrasound intensity, duration and frequency affect ultrasound energy-mediated microbubble destruction. An in vitro system was employed for their investigation. Contrast agents examined included MRX115 (DMP115), Imagent (AF0150), Levovist and Echogen. The authors noted that while no significant reduction in counts or reflectivity occurred at an ultrasound output of 0.3 W/cm², exposure to 25 W/cm² resulted in microbubble destruction, and reduction in reflectivity in all the contrast agents examined. Furthermore, the authors stated that declines in reflectivity were, increased by longer exposure to ultrasound, slower flow through the ultrasound beam, continuous rather than intermittent imaging, use of a higher pulse repetition rate, and exposure to higher frequency.

Cardiovascular safety studies were performed in dogs, rabbits, pigs, and monkeys, with the dog being the most utilized species. The dog studies RDR 98-20, 98-21, 98-22, 98-23 were generally characterized by lack of effect on measured hemodynamic parameters including mean arterial pressure, pulmonary arterial pressure, heart rate and indices of myocardial contractility. However for these studies, the doses employed were generally between multiples of 0.08-0.81 of human dose based on body surface area. Hence, how dogs will respond physiologically to equivalent amounts of DMP 115 administered to humans is not known at the present time. DMP-115 did not alter the hemodynamic profile in anesthetized rabbits dosed at between 0.073 and 5.6 MHD. Dr Grauer examined the cardiovascular safety profile of DMP-115 in anesthetized pig at doses of 0.5, 1, 2, 5 and 10µl/kg (X 0.025-0.5 MHD). The highest dose evaluated produced a transient increase in mean pulmonary pressure that returned to baseline within 8 minutes after dosing. The authors ascribed the elevation in pulmonary arterial pressure to thromboxane-mediated pulmonary hypertension caused by pulmonary intravascular